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(54) Title: DNA MOLECULES ENCODING HUMAN NHL, A DNA HELICASE

(57) Abstract: The present invention disclosed isolated nucleic acid molecules (polynucleotides) which encode NHL, a putative DNA helicase. The present invention in turn relates to recombinant vectors and recombinant hosts which contain a DNA fragment encoding NHL, substantially purified forms of associated NHL, associated mutant proteins, and methods associated with identifying compounds which modulate NHL, which will be useful in the treatment of various neoplastic disorders. Both a genomic clone containing regulatory and intron sequences, as well as the exon structure and open reading frame of human NHL are disclosed.

TITLE OF THE INVENTION

DNA MOLECULES ENCODING HUMAN NHL, A DNA HELICASE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S. provisional application 60/169,970 filed December 9, 1999.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not Applicable

REFERENCE TO MICROFICHE APPENDIX

Not Applicable

FIELD OF THE INVENTION

The present invention relates in part to isolated nucleic acid molecules (polynucleotides) which encode NHL, a putative DNA helicase. The present invention also relates to recombinant vectors and recombinant hosts which contain a DNA fragment encoding NHL, substantially purified forms of associated NHL, associated mutant proteins, and methods associated with identifying compounds which modulate NHL, which will be useful in the treatment of various neoplastic disorders, given that this gene is located at 20q13.3 and immediately adjacent to M68/DcR3, which is involved in tumor growth. Also included within the present invention is a human genomic fragment representing this portion of the human genome, along with three additional genes (M68/DcR3, SCLIP, and ARP).

BACKGROUND OF THE INVENTION

Naumovski et al. (1985, *Mol. Cell Biol.* 5:17-26; Reynolds et al. (1985 *Nucleic Acid Res* 13:2357-2372) and Weber et al. (1990 *EMBO J.* 9:1437-1447) disclose members of the RAD3/ERCC2 gene family of DNA helicases.

It is known that several chemotherapeutic agents inhibit helicases, including actinomycin C1, daunorubicin and nogalamycin (Tuteja, et al., 1997, *Biochem. Biophys. Res. Comm.* 236(3):636-640), and a prostate cancer drug, CI-958 (Lun, et al.,1998, *Cancer Chemother. Pharmacol.* 42(6):447-453). In addition, some topoisomerases have been shown to have anti-cancer activity.

Despite the identification of the aforementioned helicase-encoding genes and chemotherapeutic agents, it would be advantageous to identify additional genes which reside within chromosomal regions associated with a disease state such as cancer as well as a gene which encodes a type of protein which may be associated with that disease. The present invention addresses and meets this need by disclosing a DNA molecule encoding a DNA helicase with a chromosomal location suggestive of association with cancer.

SUMMARY OF THE INVENTION

The present invention relates to an isolated or purified nucleic acid molecule (polynucleotide) which encodes a novel mammalian DNA helicase.

The present invention also relates to an isolated nucleic acid molecule (polynucleotide) which encodes mRNA which expresses a novel human DNA helicase, NHL.

A preferred aspect of the present invention relates to an isolated or purified DNA molecule which encodes human NHL, the nucleotide sequence as set forth in Figure 1A-B and SEQ ID NO:1.

The present invention also relates to biologically active fragments or mutants of SEQ ID NO:1 which encode a mRNA molecule expressing a novel DNA helicase, NHL. Any such biologically active fragment and/or mutant will encode either a protein or protein fragment which at least substantially mimics the biological properties of the human NHL protein disclosed herein in Figure 2 and as set forth as SEQ ID NO:2. Any such polynucleotide includes but is not necessarily limited to

nucleotide substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal truncations such that these mutations encode mRNA which express a functional NHL protein in a host cell, so as to be useful for screening for agonists and/or antagonists of NHL activity.

The present invention also relates to recombinant vectors and recombinant hosts, both prokaryotic and eukaryotic, which contain the substantially purified nucleic acid molecules disclosed throughout this specification.

The present invention also relates to a substantially purified form of a human NHL protein which comprises the amino acid sequence disclosed in Figure 2 and set forth as SEQ ID NO:2.

A preferred aspect of this portion of the present invention is a NHL protein which consists of the amino acid sequence disclosed in Figure 2 and set forth as SEQ ID NO:2.

Another preferred aspect of the present invention relates to a substantially purified NHL protein, preferably a human NHL protein, obtained from a recombinant host cell containing a DNA expression vector comprises a nucleotide sequence as set forth in SEQ ID NO:1 and expresses the respective NHL protein. It is especially preferred is that the recombinant host cell be a eukaryotic host cell, such as a mammalian cell line.

The present invention also relates to biologically active fragments and/or mutants of a NHL protein comprising the amino acid sequence as set forth in SEQ ID NO:2, including but not necessarily limited to amino acid substitutions, deletions, additions, amino terminal truncations and carboxy-terminal truncations such that these mutations provide for proteins or protein fragments of diagnostic, therapeutic or prophylactic use and would be useful for screening for selective modulators, including but not limited to agonists and/or antagonists for human NHL pharmacology.

A preferred aspect of the present invention is disclosed in Figure 2 and is set forth as SEQ ID NO:2, a respective amino acid sequence which encodes human NHL. Characterization of one or more of these DNA helicase-like proteins allows for screening methods to identify novel NHL modulators that may be useful in the treatment of human neoplastic disorders. The modulators selected through such screening and selection protocols may be used alone or in conjunction with other cancer therapies. As noted above, heterologous expression of a NHL protein will allow the pharmacological analysis of compounds which modulate NHL activity and

hence may be useful in various cancer therapies. To this end, heterologous cell lines expressing a NHL protein can be used to establish functional or binding assays to identify novel NHL modulators.

The present invention also relates to polyclonal and monoclonal antibodies raised in response to either the NHL or a biologically active fragment of NHL.

The present invention relates to transgenic mice comprising altered genotypes and phenotypes in relation to NHL and its *in vivo* activity.

The present invention also relates to NHL fusion constructs, including but not limited to fusion constructs which express a portion of the NHL protein linked to various markers, including but in no way limited to GFP (Green fluorescent protein), the MYC epitope, and GST. Any such fusion constructs may be expressed in the cell line of interest and used to screen for NHL modulators.

Therefore, the present invention relates to methods of expressing mammalian NHL, and preferably human NHL, biological equivalents disclosed herein, assays employing these gene products, recombinant host cells which comprise DNA constructs which express these proteins, and compounds identified through these assays which act as agonists or antagonists of NHL activity.

The present invention also relates to the isolated genomic sequence which comprises SEQ ID NO:1, a 115 kb genomic fragment set forth herein as SEQ ID NO:3. As especially preferred aspect of this portion of the invention is the region of the genomic fragment of SEQ ID NO:3 which comprises the regulatory and coding regions of human NHL, as well as intervening sequences (introns). This 115 kb fragment contains at least the coding region of four genes, NHL, M68/DcR3, SCLIP and ARP. As discussed herein, it has been shown that this region of chromosome 20 is associated with tumor growth. Therefore, an aspect of this invention also comprises the use of one or more regions of this 115 kb genomic sequence to identify compounds which up or downregulate expression of one or more of the genes localized within this 115 kb region, wherein this up or down regulation results in an interference of tumor growth. For example, a transcription element of one of these four genes may be responsible for M68/DcR3 (and/or NHL) overexpression in tumors, and if M68 or NHL overexpression in tumors has a caustic role, blockage of M68/DcR3 or NHL overexpression in tumors by interfering with this transcription site will be useful.

It is an object of the present invention to provide an isolated nucleic acid molecule (e.g., SEQ ID NO:1) which encodes novel form of human NHL, or fragments, mutants or derivatives of human NHL as set forth in Figure 2 and SEQ ID NO:2. Any such polynucleotide includes but is not necessarily limited to nucleotide substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal truncations such that these mutations encode mRNA which express a protein or protein fragment of diagnostic, therapeutic or prophylactic use and would be useful for screening for selective modulators of human NHL activity.

It is a further object of the present invention to provide the mammalian, and especially human, NHL proteins or protein fragments encoded by the nucleic acid molecules referred to in the preceding paragraph.

It is a further object of the present invention to provide recombinant vectors and recombinant host cells which comprise a nucleic acid sequence encoding mammalian, and especially human, NHL protein and biological equivalent thereof.

It is an object of the present invention to provide a substantially purified form of human NHL, as set forth in Figure 2 and SEQ ID NO:2.

Is another object of the present invention to provide a substantially purified recombinant form of a NHL protein which has been obtained from a recombinant host cell transformed or transfected with a DNA expression vector which comprises and appropriately expresses a complete open reading frame as set forth in SEQ ID NO:1, resulting in a functional, processed form of NHL. It is especially preferred is that the recombinant host cell be a eukaryotic host cell, such as a mammalian cell line.

It is an object of the present invention to provide for biologically active fragments and/or mutants of mammalian, and especially human, NHL, such as set forth in SEQ ID NO:2, including but not necessarily limited to amino acid substitutions, deletions, additions, amino terminal truncations and carboxy-terminal truncations such that these mutations provide for proteins or protein fragments of diagnostic, therapeutic and/or prophylactic use.

It is also an object of the present invention to use NHL proteins or biological equivalent to screen for modulators, preferably selective modulators, of human NHL activity. Any such compound may be useful in screening for and selecting compounds active against human neoplastic disorders.

As used herein, "substantially free from other nucleic acids" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of

other nucleic acids. Thus, a human NHL DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-NHL nucleic acids. Whether a given NHL DNA preparation is substantially free from other nucleic acids can be determined by such conventional techniques of assessing nucleic acid purity as, *e.g.*, agarose gel electrophoresis combined with appropriate staining methods, *e.g.*, ethidium bromide staining, or by sequencing.

As used herein, "substantially free from other proteins" or "substantially purified" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins. Thus, a NHL protein preparation that is substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-NHL proteins. Whether a given NHL protein preparation is substantially free from other proteins can be determined by such conventional techniques of assessing protein purity as, *e.g.*, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, *e.g.*, silver staining or immunoblotting. As used interchangeably with the terms "substantially free from other proteins" or "substantially purified", the terms "isolated NHL protein" or "purified NHL protein" also refer to NHL protein that has been isolated from a natural source. Use of the term "isolated" or "purified" indicates that NHL protein has been removed from its normal cellular environment. Thus, an isolated NHL protein may be in a cell-free solution or placed in a different cellular environment from that in which it occurs naturally. The term isolated does not imply that an isolated NHL protein is the only protein present, but instead means that an isolated NHL protein is substantially free of other proteins and non-amino acid material (*e.g.*, nucleic acids, lipids, carbohydrates) naturally associated with the NHL protein *in vivo*. Thus, a NHL protein that is recombinantly expressed in a prokaryotic or eukaryotic cell and substantially purified from this host cell which does not naturally (*i.e.*, without intervention) express this protein is of course "isolated NHL protein" under any circumstances referred to herein. As noted above, a NHL protein preparation that is an isolated or purified NHL protein will be substantially free from other proteins will contain, as a percent of its total protein, no more than 10%,

preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-NHL proteins.

As used interchangeably herein, "functional equivalent" or "biologically active equivalent" means a protein which does not have exactly the same amino acid sequence as naturally occurring NHL, due to alternative splicing, deletions, mutations, substitutions, or additions, but retains substantially the same biological activity as NHL. Such functional equivalents will have significant amino acid sequence identity with naturally occurring NHL and genes and cDNA encoding such functional equivalents can be detected by reduced stringency hybridization with a DNA sequence encoding naturally occurring NHL. For example, a naturally occurring NHL disclosed herein comprises the amino acid sequence shown as SEQ ID NO:2 and is encoded by SEQ ID NO:1. A nucleic acid encoding a functional equivalent has at least about 50% identity at the nucleotide level to SEQ ID NO:1.

As used herein, "a conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (e.g., arginine for lysine; glutamic acid for aspartic acid).

As used herein, the term "mammalian" will refer to any mammal, including a human being.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A-B shows the nucleotide sequence which comprises the open reading frame which encodes human NHL, the nucleotide sequence set forth as SEQ ID NO:1. The initiating Met residue (ATG) and the stop codon (TAG) are underlined.

Figure 2 shows the amino acid sequence of human NHL as set forth in SEQ ID NO:2.

Figure 3 shows the alignment of amino acid sequences of human NHL to ERCC2/RAD3 gene family members. Rep D (*Dictyosteliem discoideum*); RAD 3 (*S. cerevisiae*); RAD15 (*S. pombe*) and XP_GroupD (*Homo sapien*).

Figure 4 shows Northern analysis of NHL expression in multi-human tissues.

Figure 5A-B show the genomic structure of the NHL gene (Figure 5A) and the entire 115 kb genomic region (Figure 5B) containing the NHL, M68/DcR3, SCLIP

and ARP genes.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to an isolated or purified nucleic acid molecule (polynucleotide) which encodes a novel mammalian DNA helicase. An especially preferred aspect of this invention relates to an isolated nucleic acid molecule (polynucleotide) which encodes mRNA which expresses a novel human DNA helicase, NHL.

The gene M68/DcR3 is a secreted TNFR member that is overexpressed in a number of human tumors. M68/DcR3 is located at 20q13.3, a known site that is associated with frequent gene amplification in cancer. M68/DcR3 protein binds to FASL and inhibit FAS mediated apoptosis. Thus, genes tightly linked to M68/DcR3 may be coregulated (e.g. co overexpressed and/or amplified in tumors). During the course of cloning the genomic M68/DcR3 fragment and identifying genes that are linked to M68/DcR3 at 20q13.3, three genes, including a novel gene that is similar to the Rad3/ERCC2 helicase family, were identified (termed NHL) in the immediately adjacent (overlapping) region. Given NHL's chromosomal location and the frequent association of DNA helicases with human genetic disorders (mutations in DNA helicases have been found associated with multiple diseases, including xeroderma pigmentosum, Cockayne's syndrome, Bloom's syndrome, and Werner's syndrome), NHL is a candidate for contribution to certain human neoplastic disorders. To this end, the genomic clone for this gene is disclosed and the complete sequence is determined. The transcript was identified through exon prediction using GRAIL2 and sequence alignment to a contiguous 4.5 kilobase region of chromosome 4 (88% sequence identity). The complete exon structure of NHL was subsequently confirmed by RT-PCR analysis. Multiple sequence alignment of NHL to known helicases showed that NHL contains all the seven critical helicase domains. BLAST analysis of the predicted 1,219 amino acid sequence revealed an approximately 26% sequence identity and 48% sequence similarity to the RAD3/ERCC2 gene family of DNA helicases (Naumovski et al., 1985 *Mol. Cell Biol.* 5:17-26; Reynolds et al., 1985 *Nucleic Acid Res* 13:2357-72; Weber et al., 1990 *EMBO J.* 9:1437-1447). The mRNA expression pattern of NHL was also examined in multiple human tissues. Radiation hybrid chromosomal mapping reconfirms that it is linked to M68/DcR3 locus.

A preferred aspect of the present invention relates to an isolated or purified DNA molecule which encodes human NHL, the nucleotide sequence as set forth in Figure 1A-B and SEQ ID NO:1, which is as follows:

AGTCAGCCCT GCTGCCAGCC AGTGCCGGGT GCTGGGGACT CAGGGAGGCC CGCCGGGACC
ACTGCGGGAC AGTGAGCCGA GCAGAAGCTG GAACGCAGGA GAGGAAGGAG AGGGGGCGGT
CAGGGCTCTC AGGAGCCGGG TCCTGGGCAA GGCGCAGCCG TTTTCAAATT TTCAGGAAAG
CGGTCGGCTC ACACTCGAGC AGTAAAAAGA TGCCTCTGGG GAGGAGGCCC GTGCAGCTCT
CCGGGCAATG GTGGTGGCTC GGCCTAGAGA GGCGGTAGTG GAACGCAGAC CCTGGTGGGG
GAATGACATC AAGGGAGGAG ACGGGCGGGA CCCCAGATTT CTGCCTGTGG GCGATGGAAG
TGAGGTTCAC TGGCCAGCGG AGCCGGACAC AGAACGCGCA AAACGCCGTG TAGGCCTGGA
GGAGCCGAAG AGCAGGCGGA CCCCCTCCGC GGGGGAACAG TTTCCGCCGG GAGCACAAAG
CAACGGACCG GAAGTGGGGG GCGGAAGTGC AGTGGGCTCA GCGCCGACTG CGCGCCTCTG
CCCGCGAAAA CTCTGAGCTG GCTGACAGCT GGGGACGGGT GGCGGCCCTC GACTGGAGTC
GGTTGAGTTC CTGAGGGACC CCGGTTCTGG AAGGTTCGCC GCGGAGACAA GTGAGCAGTC
TGTGCCATAG GGATTCTCGA AGAGAACAGC GTTGTGTCCC AGTGCACATG CTCGCATCGC
TTACCAGGAG TGCCCGAGAC CCTAAGATGT TCGGAGTGGT TTTTTCGCAC AGACCCGAAT
AGCCTGCCCC TCAGCCACGC TCTGTGCCCT TCTGAGAACA GGCTGATATG CCCAAGATAG
TCCTGAATGG TGTGACCGTA GACTTCCCTT TCCAGCCCTA CAAATGCCAA CAGGAGTACA
TGACCAAGGT CCTGGAATGT CTGCAGCAGA AGGTGAATGG CATCCTGGAG AGCCCTACGG
GTACAGGGAA GACGCTGTGC CTGCTGTGCA CCACGCTGGC CTGGCGAGAA CACCTCCGAG
ACGGCATCTC TGCCCGCAAG ATTGCCGAGA GGGCGCAAGG AGAGCTTTTC CCGGATCGGG
CCTTGTCATC CTGGGGCAAC GCTGCTGCTG CTGCTGGAGA CCCCATAGCT TGCTACACGG
ACATCCCAAA GATTATTTAC GCCTCCAGGA CCCACTCGCA ACTCACACAG GTCATCAACG
AGCTTCGGAA CACCTCCTAC CGGCCTAAGG TGTGTGTGCT GGGCTCCCGG GAGCAGCTGT
GCATCCATCC TGAGGTGAAG AAACAAGAGA GTAACCATCT ACAGATCCAC TTGTGCCGTA
AGAAGGTGGC AAGTCGCTCC TGTCATTTCT ACAACAACGT AGAAGAAAAA AGCCTGGAGC
AGGAGCTGGC CAGCCCCATC CTGGACATTG AGGACTTGGT CAAGAGCGGA AGCAAGCACA
GGGTGTGCCC TTACTACCTG TCCCGGAACC TGAAGCAGCA AGCCGACATC ATATTCATGC
CGTACAATTA CTTGTTGGAT GCCAAGAGCC GCAGAGCACA CAACATTGAC CTGAAGGGGA
CAGTCGTGAT CTTTGACGAA GCTCACAACG TGGAGAAGAT GTGTGAAGAA TCGGCATCCT
TTGACCTGAC TCCCCATGAC CTGGCTTCAG GACTGGACGT CATAGACCAG GTGCTGGAGG
AGCAGACCAA GGCAGCGCAG CAGGGTGAGC CCCACCCGGA GTTCAGCGCG GACTCCCCCA
GCCCAGGGCT GAACATGGAG CTGGAAGACA TTGCAAAGCT GAAGATGATC CTGCTGCGCC
TGGAGGGGGC CATCGATGCT GTTGAGCTGC CTGGAGACGA CAGCGGTGTC ACCAAGCCAG

GGAGCTACAT CTTTGAGCTG TTTGCTGAAG CCCAGATCAC GTTTCAGACC AAGGGCTGCA
TCCTGGACTC GCTGGACCAG ATCATCCAGC ACCTGGCAGG ACGTGCTGGA GTGTTCACCA
ACACGGCCGG ACTGCAGAAG CTGGCGGACA TTATCCAGAT TGTGTTCAGT GTGGACCCCT
CCGAGGGCAG CCCTGGTTCC CCAGCAGGGC TGGGGGCCTT ACAGTCCTAT AAGGTGCACA
TCCATCCTGA TGCTGGTCAC CGGAGGACGG CTCAGCGGTC TGATGCCTGG AGCACCACTG
CAGCCAGAAA GCGAGGGAAG GTGCTGAGCT ACTGGTGCTT CAGTCCCGGC CACAGCATGC
ACGAGCTGGT CCGCCAGGGC GTCCGCTCCC TCATCCTTAC CAGCGGCACG CTGGCCCCGG
TGTCCTCCTT TGCTCTGGAG ATGCAGATCC CTTTCCCAGT CTGCCTGGAG AACCCACACA
TCATCGACAA GCACCAGATC TGGGTGGGGG TCGTCCCCAG AGGCCCCGAT GGAGCCCAGT
TGAGCTCCGC GTTTGACAGA CGGTTTTCCG AGGAGTGCTT ATCCTCCCTG GGGAAGGCTC
TGGGCAACAT CGCCCGCGTG GTGCCCTATG GGCTCCTGAT CTTCTTCCCT TCCTATCCTG
TCATGGAGAA GAGCCTGGAG TTCTGGCGGG CCCGCGACTT GGCCAGGAAG ATGGAGGCGC
TGAAGCCGCT GTTTGTGGAG CCCAGGAGCA AAGGCAGCTT CTCCGAGACC ATCAGTGCTT
ACTATGCAAG GGTTGCCGCC CCTGGGTCCA CCGGCGCCAC CTTCCTGGCG GTCTGCCGGG
GCAAGGCCAG CGAGGGGCTG GACTTCTCAG ACACGAATGG CCGTGGTGTG ATTGTCACGG
GCCTCCCGTA CCCCCCACGC ATGGACCCCC GGGTTGTCCT CAAGATGCAG TTCCTGGATG
AGATGAAGGG CCAGGGTGGG GCTGGGGGCC AGTTCCTCTC TGGGCAGGAG TGGTACCGGC
AGCAGGCGTC CAGGGCTGTG AACCAGGCCA TCGGGCGAGT GATCCGGCAC CGCCAGGACT
ACGGAGCTGT CTTCCTCTGT GACCACAGGT TCGCCTTTGC CGACGCAAGA GCCCAACTGC
CCTCCTGGGT GCGTCCCCAC GTCAGGGTGT ATGACAACTT TGGCCATGTC ATCCGAGACG
TGGCCCAGTT CTTCCGTGTT GCCGAGCGAA CTATGCCAGC GCCGGCCCCC CGGGCTACAG
CACCCAGTGT GCGTGGAGAA GATGCTGTCA GCGAGGCCAA GTCGCCTGGC CCCTTCTTCT
CCACCAGGAA AGCTAAGAGT CTGGACCTGC ATGTCCCCAG CCTGAAGCAG AGGTCCTCAG
GGTCACCAGC TGCCGGGGAC CCCGAGAGTA GCCTGTGTGT GGAGTATGAG CAGGAGCCAG
TTCCTGCCCG GCAGAGGCCC AGGGGGCTGC TGGCCGCCCT GGAGCACAGC GAACAGCGGG
CGGGGAGCCC TGGCGAGGAG CAGGCCCACA GCTGCTCCAC CCTGTCCCTC CTGTCTGAGA
AGAGGCCGGC AGAAGAACCG CGAGGAGGGA GGAAGAAGAT CCGGCTGGTC AGCCACCCGG
AGGAGCCCGT GGCTGGTGCA CAGACGGACA GGGCCAAGCT CTTCATGGTG GCCGTGAAGC
AGGAGTTGAG CCAAGCCAAC TTTGCCACCT TCACCCAGGC CCTGCAGGAC TACAAGGGTT
CCGATGACTT CGCCGCCCTG GCCGCCTGTC TCGGCCCCCT CTTTGCTGAG GACCCCAAGA
AGCACAACCT GCTCCAAGGC TTCTACCAGT TTGTGCGGCC CCACCATAAG CAGCAGTTTG
AGGAGGTCTG TATCCAGCTG ACAGGACGAG GCTGTGGCTA TCGGCCTGAG CACAGCATTC
CCCGAAGGCA GCGGGCACAG CCGGTCCTGG ACCCCACTGG AAGAACGGCG CCGGATCCCA
AGCTGACCGT GTCCACGGCT GCAGCCCAGC AGCTGGACCC CCAAGAGCAC CTGAACCAGG

GCAGGCCCCA CCTGTCGCCC AGGCCACCCC CAACAGGAGA CCCTGGCAGC CAACCACAGT
GGGGGTCTGG AGTGCCCAGA GCAGGGAAGC AGGGCCAGCA CGCCGTGAGC GCCTACCTGG
CTGATGCCCG CAGGGCCCTG GGGTCCGCGG GCTGTAGCCA ACTCTTGGCA GCGCTGACAG
CCTATAAGCA AGACGACGAC CTCGACAAGG TGCTGGCTGT GTTGGCCGCC CTGACCACTG
CAAAGCCAGA GGACTTCCCC CTGCTGCACA GGTTCAGCAT GTTTGTGCGT CCACACCACA
AGCAGCGCTT CTCACAGACG TGCACAGACC TGACCGGCCG GCCCTACCCG GGCATGGAGC
CACCGGGACC CCAGGAGGAG AGGCTTGCCG TGCCTCCTGT GCTTACCCAC AGGGCTCCCC
AACCAGGCCC CTCACGGTCC GAGAAGACCG GGAAGACCCA GAGCAAGATC TCGTCCTTCC
TTAGACAGAG GCCAGCAGGG ACTGTGGGGG CGGGCGGTGA GGATGCAGGT CCCAGCCAGT
CCTCAGGACC TCCCCACGGG CCTGCAGCAT CTGAGTGGGG CCTCTAGGAT GTGCCCAGCC
TGCCACACCG CCTCCAGGAA GCAGAGCGTC ATGCAGGTCT TCTGGCCAGA GCCCCAGTGA
GTGCCCACGG AGGCCCCCAG CACACCCAAC GTGGCTTGAT CACCTGCCTG TCCAGCTCTG
GTGGGCCAAG AACCCACCCA ACAGAATAGG CCAGCCCATG CCAGCCGGCT TGGCCCGCTG
CAGGCCTCAG GCAGGCGGGG CCCATGGTTG GTCCCTGCGG TGGGACCGGA TCTGGGCCTG
CCTCTGAGAA GCCCTGAGCT ACCTTGGGGT CTGGGGTGGG TTTCTGGGAA AGTGCTTCCC
CAGAACTTCC CTGGCTCCTG GCCTGTGAGT GGTGCCACAG GGGCACCCCA GCTGAGCCCC
TCACCGGGAA GGAGGAGACC CCCGTGGGCA CGTGTCCACT TTTAATCAGG GGACAGGGCT
CTCTAATAAA GCTGCTGGCA GTGCCC (SEQ ID NO:1).

The above-exemplified isolated DNA molecule shown in Figure 1A-B and SEQ ID NO:1 comprise 4946 nucleotides, with an initiating Met at nucleotides 828-830 and a "TAG" termination codon at nucleotides 4585-4587. The initiating Met and TAG termination codon are underlined.

The present invention also relates to biologically active fragments or mutants of SEQ ID NO:1 which encode a mRNA molecule expressing a novel DNA helicase, NHL. Any such biologically active fragment and/or mutant will encode either a protein or protein fragment which at least substantially mimics the biological properties of the human NHL protein disclosed herein in Figure 2 and as set forth as SEQ ID NO:2. Any such polynucleotide includes but is not necessarily limited to nucleotide substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal truncations such that these mutations encode mRNA which express a functional NHL protein in a host cell, so as to be useful for screening for agonists and/or antagonists of NHL activity.

The isolated nucleic acid molecules of the present invention may include a deoxyribonucleic acid molecule (DNA), such as genomic DNA and complementary

DNA (cDNA), which may be single (coding or noncoding strand) or double stranded, as well as synthetic DNA, such as a synthesized, single stranded polynucleotide. The isolated nucleic acid molecule of the present invention may also include a ribonucleic acid molecule (RNA).

The present invention also relates to recombinant vectors and recombinant hosts, both prokaryotic and eukaryotic, which contain the substantially purified nucleic acid molecules disclosed throughout this specification.

The degeneracy of the genetic code is such that, for all but two amino acids, more than a single codon encodes a particular amino acid. This allows for the construction of synthetic DNA that encodes the NHL protein where the nucleotide sequence of the synthetic DNA differs significantly from the nucleotide sequence of SEQ ID NO:1 but still encodes the same NHL protein as SEQ ID NO:2. Such synthetic DNAs are intended to be within the scope of the present invention. If it is desired to express such synthetic DNAs in a particular host cell or organism, the codon usage of such synthetic DNAs can be adjusted to reflect the codon usage of that particular host, thus leading to higher levels of expression of the NHL protein in the host. In other words, this redundancy in the various codons which code for specific amino acids is within the scope of the present invention. Therefore, this invention is also directed to those DNA sequences which encode RNA comprising alternative codons which code for the eventual translation of the identical amino acid, as shown below:

A=Ala=Alanine: codons GCA, GCC, GCG, GCU
C=Cys=Cysteine: codons UGC, UGU
D=Asp=Aspartic acid: codons GAC, GAU
E=Glu=Glutamic acid: codons GAA, GAG
F=Phe=Phenylalanine: codons UUC, UUU
G=Gly=Glycine: codons GGA, GGC, GGG, GGU
H=His =Histidine: codons CAC, CAU
I=Ile =Isoleucine: codons AUA, AUC, AUU
K=Lys=Lysine: codons AAA, AAG
L=Leu=Leucine: codons UUA, UUG, CUA, CUC, CUG, CUU
M=Met=Methionine: codon AUG
N=Asp=Asparagine: codons AAC, AAU
P=Pro=Proline: codons CCA, CCC, CCG, CCU

Q=Gln=Glutamine: codons CAA, CAG

R=Arg=Arginine: codons AGA, AGG, CGA, CGC, CGG, CGU

S=Ser=Serine: codons AGC, AGU, UCA, UCC, UCG, UCU

T=Thr=Threonine: codons ACA, ACC, ACG, ACU

V=Val=Valine: codons GUA, GUC, GUG, GUU

W=Trp=Tryptophan: codon UGG

Y=Tyr=Tyrosine: codons UAC, UAU

Therefore, the present invention discloses codon redundancy which may result in differing DNA molecules expressing an identical protein. For purposes of this specification, a sequence bearing one or more replaced codons will be defined as a degenerate variation. Also included within the scope of this invention are mutations either in the DNA sequence or the translated protein which do not substantially alter the ultimate physical properties of the expressed protein. For example, substitution of valine for leucine, arginine for lysine, or asparagine for glutamine may not cause a change in functionality of the polypeptide.

It is known that DNA sequences coding for a peptide may be altered so as to code for a peptide having properties that are different than those of the naturally occurring peptide. Methods of altering the DNA sequences include but are not limited to site directed mutagenesis. Examples of altered properties include but are not limited to changes in the affinity of an enzyme for a substrate or a receptor for a ligand.

The present invention also relates to recombinant vectors and recombinant hosts, both prokaryotic and eukaryotic, which contain the substantially purified nucleic acid molecules disclosed throughout this specification. The nucleic acid molecules of the present invention encoding a NHL protein, in whole or in part, can be linked with other DNA molecules, i.e, DNA molecules to which the NHL coding sequence are not naturally linked, to form "recombinant DNA molecules" which encode a respective NHL protein. The novel DNA sequences of the present invention can be inserted into vectors which comprise nucleic acids encoding NHL or a functional equivalent. These vectors may be comprised of DNA or RNA; for most cloning purposes DNA vectors are preferred. Typical vectors include plasmids, modified viruses, bacteriophage, cosmids, yeast artificial chromosomes, and other forms of episomal or integrated DNA that can encode a NHL protein. It is well within

the purview of the skilled artisan to determine an appropriate vector for a particular gene transfer or other use.

Included in the present invention are DNA sequences that hybridize to SEQ ID NO:1 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows: Prehybridization of filters containing DNA is carried out for 2 hours to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 μg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hrs at 65°C in prehybridization mixture containing 100 μg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 37°C for 1 hr in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography. Other procedures using conditions of high stringency would include either a hybridization step carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, e.g., Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

The present invention also relates to a substantially purified form of a human NHL protein which comprises the amino acid sequence (1219 amino acid residues) disclosed in Figure 2 and set forth as SEQ ID NO:2. A preferred aspect of this portion of the present invention is a NHL protein which consists of the amino acid sequence disclosed in Figure 2 and set forth as SEQ ID NO:2, as follows:

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MPKIVLNGVT VDFPFQPYKC QQEYMTKVLE CLQQKVNGIL ESPTGTGKTL CLLCTTLAWR
EHLRDGISAR KIAERAQGEL FPDRALSSWG NAAAAAGDPI ACYTDIPKII YASRTHSQLT
QVINELRNTS YRPKVCVLGS REQLCIHPEV KKQESNHLQI HLCRKKVASR SCHFYNNVEE
KSLEQELASP ILDIEDLVKS GSKHRVCPYY LSRNLKQQAD IIFMPYNYLL DAKSRRAHNI
DLKGTVVIFD EAHNVEKMCE ESASFDLTPH DLASGLDVID QVLEEQTKAA QQGEPHPEFS
ADSPSPGLNM ELEDIAKLKM ILLRLEGAID AVELPGDDSG VTKPGSYIFE LFAEAQITFQ
TKGCILDSLD QIIQHLAGRA GVFTNTAGLQ KLADIIQIVF SVDPSEGSPG SPAGLGALQS
YKVHIHPDAG HRRTAQRSDA WSTTAARKRG KVLSYWCFSP GHSMHELVRQ GVRSLILTSG
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TLAPVSSFAL EMQIPFPVCL ENPHIIDKHQ IWVGVVPRGP DGAQLSSAFD RRFSEECLSS LGKALGNIAR VVPYGLLIFF PSYPVMEKSL EFWRARDLAR KMEALKPLFV EPRSKGSFSE TISAYYARVA APGSTGATFL AVCRGKASEG LDFSDTNGRG VIVTGLPYPP RMDPRVVLKM QFLDEMKGQG GAGGQFLSGQ EWYRQQASRA VNQAIGRVIR HRQDYGAVFL CDHRFAFADA RAQLPSWVRP HVRVYDNFGH VIRDVAQFFR VAERTMPAPA PRATAPSVRG EDAVSEAKSP GPFFSTRKAK SLDLHVPSLK QRSSGSPAAG DPESSLCVEY EQEPVPARQR PRGLLAALEH SEQRAGSPGE EQAHSCSTLS LLSEKRPAEE PRGGRKKIRL VSHPEEPVAG AQTDRAKLFM VAVKQELSQA NFATFTQALQ DYKGSDDFAA LAACLGPLFA EDPKKHNLLQ GFYQFVRPHH KQQFEEVCIQ LTGRGCGYRP EHSIPRRQRA QPVLDPTGRT APDPKLTVST AAAQQLDPQE HLNQGRPHLS PRPPPTGDPG SQPQWGSGVP RAGKQGQHAV SAYLADARRA LGSAGCSQLL AALTAYKQDD DLDKVLAVLA ALTTAKPEDF PLLHRFSMFV RPHHKQRFSQ TCTDLTGRPY PGMEPPGPQE ERLAVPPVLT HRAPQPGPSR SEKTGKTQSK ISSFLRQRPA GTVGAGGEDA GPSQSSGPPH GPAASEWGL* (SEQ ID NO:2).

The present invention also relates to biologically active fragments and/or mutants of the human NHL protein comprising the amino acid sequence as set forth in SEQ ID NO:2, including but not necessarily limited to amino acid substitutions, deletions, additions, amino terminal truncations and carboxy-terminal truncations such that these mutations provide for proteins or protein fragments of diagnostic, therapeutic or prophylactic use and would be useful for screening for agonists and/or antagonists of NHL function.

Another preferred aspect of the present invention relates to a substantially purified, fully processed NHL protein obtained from a recombinant host cell containing a DNA expression vector which comprises a nucleotide sequence as set forth in SEQ ID NO:1 and expresses the human NHL protein. It is especially preferred is that the recombinant host cell be a eukaryotic host cell, such as a mammalian cell line.

As with many proteins, it is possible to modify many of the amino acids of NHL protein and still retain substantially the same biological activity as the wild type protein. Thus this invention includes modified NHL polypeptides which have amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as a respective, corresponding NHL. It is generally accepted that single amino acid substitutions do not usually alter the biological activity of a protein (see, e.g., *Molecular Biology of the Gene*, Watson *et al.*, 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells, 1989,

Science 244:1081-1085). Accordingly, the present invention includes a polypeptide where one amino acid substitution has been made in SEQ ID NO:2 wherein the polypeptide still retains substantially the same biological activity as a corresponding NHL protein. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ ID NO:2 wherein the polypeptide still retains substantially the same biological activity as a corresponding NHL protein. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions.

One skilled in the art would also recognize that polypeptides that are functional equivalents of NHL and have changes from the NHL amino acid sequence that are small deletions or insertions of amino acids could also be produced by following the same guidelines, (i.e, minimizing the differences in amino acid sequence between NHL and related proteins. Small deletions or insertions are generally in the range of about 1 to 5 amino acids). The effect of such small deletions or insertions on the biological activity of the modified NHL polypeptide can easily be assayed by producing the polypeptide synthetically or by making the required changes in DNA encoding NHL and then expressing the DNA recombinantly and assaying the protein produced by such recombinant expression.

The present invention also includes truncated forms of NHL which contain the region comprising the active site of the enzyme. Such truncated proteins are useful in various assays described herein, for crystallization studies, and for structure-activity-relationship studies.

The present invention also relates to isolated nucleic acid molecules which are fusion constructions expressing fusion proteins useful in assays to identify compounds which modulate wild-type NHL activity, as well as generating antibodies against NHL. One aspect of this portion of the invention includes, but is not limited to, glutathione S-transferase (GST)-NHL fusion constructs. Recombinant GST-NHL fusion proteins may be expressed in various expression systems, including *Spodoptera frugiperda* (Sf21) insect cells (Invitrogen) using a baculovirus expression vector (pAcG2T, Pharmingen). Another aspect involves NHL fusion constructs linked to various markers, including but not limited to GFP (Green fluorescent protein), the MYC epitope, and GST. Again, any such fusion constructs may be expressed in the cell line of interest and used to screen for modulators of one or more of the NHL proteins disclosed herein.

Any of a variety of procedures may be used to clone NHL. These methods include, but are not limited to, (1) a RACE PCR cloning technique (Frohman, et al., 1988, *Proc. Natl. Acad. Sci. USA* 85: 8998-9002). 5′ and/or 3′ RACE may be performed to generate a full-length cDNA sequence. This strategy involves using gene-specific oligonucleotide primers for PCR amplification of NHL cDNA. These gene-specific primers are designed through identification of an expressed sequence tag (EST) nucleotide sequence which has been identified by searching any number of publicly available nucleic acid and protein databases; (2) direct functional expression of the NHL cDNA following the construction of a NHL-containing cDNA library in an appropriate expression vector system; (3) screening a NHL-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a labeled degenerate oligonucleotide probe designed from the amino acid sequence of the NHL protein; (4) screening a NHL-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a partial cDNA encoding the NHL protein. This partial cDNA is obtained by the specific PCR amplification of NHL DNA fragments through the design of degenerate oligonucleotide primers from the amino acid sequence known for other kinases which are related to the NHL protein; (5) screening a NHL-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a partial cDNA or oligonucleotide with homology to a mammalian NHL protein. This strategy may also involve using gene-specific oligonucleotide primers for PCR amplification of NHL cDNA identified as an EST as described above; or (6) designing 5′ and 3′ gene specific oligonucleotides using SEQ ID NO: 1 as a template so that either the full-length cDNA may be generated by known RACE techniques, or a portion of the coding region may be generated by these same known RACE techniques to generate and isolate a portion of the coding region to use as a probe to screen one of numerous types of cDNA and/or genomic libraries in order to isolate a full-length version of the nucleotide sequence encoding NHL.

It is readily apparent to those skilled in the art that other types of libraries, as well as libraries constructed from other cell types-or species types, may be useful for isolating a NHL-encoding DNA or a NHL homologue. Other types of libraries include, but are not limited to, cDNA libraries derived from other cells.

It is readily apparent to those skilled in the art that suitable cDNA libraries may be prepared from cells or cell lines which have NHL activity. The selection of cells or cell lines for use in preparing a cDNA library to isolate a cDNA encoding

NHL may be done by first measuring cell-associated NHL activity using any known assay available for such a purpose.

Preparation of cDNA libraries can be performed by standard techniques well known in the art. Well known cDNA library construction techniques can be found for example, in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. Complementary DNA libraries may also be obtained from numerous commercial sources, including but not limited to Clontech Laboratories, Inc. and Stratagene.

It is also readily apparent to those skilled in the art that DNA encoding NHL may also be isolated from a suitable genomic DNA library. Construction of genomic DNA libraries can be performed by standard techniques well known in the art. Well known genomic DNA library construction techniques can be found in Sambrook, et al., *supra*. One may prepare genomic libraries, especially in P1 artificial chromosome vectors, from which genomic clones containing the NHL gene can be isolated, using probes based upon the NHL nucleotide sequences disclosed herein. Methods of preparing such libraries are known in the art (Ioannou et al., 1994, *Nature Genet.* 6:84-89).

In order to clone a NHL gene by one of the preferred methods, the amino acid sequence or DNA sequence of a NHL or a homologous protein may be necessary. To accomplish this, a respective NHL protein may be purified and the partial amino acid sequence determined by automated sequenators. It is not necessary to determine the entire amino acid sequence, but the linear sequence of two regions of 6 to 8 amino acids can be determined for the PCR amplification of a partial NHL DNA fragment. Once suitable amino acid sequences have been identified, the DNA sequences capable of encoding them are synthesized. Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid, and therefore, the amino acid sequence can be encoded by any of a set of similar DNA oligonucleotides. Only one member of the set will be identical to the NHL sequence but others in the set will be capable of hybridizing to NHL DNA even in the presence of DNA oligonucleotides with mismatches. The mismatched DNA oligonucleotides may still sufficiently hybridize to the NHL DNA to permit identification and isolation of NHL encoding DNA. Alternatively, the nucleotide sequence of a region of an expressed sequence may be identified by searching one or more available genomic databases. Gene-specific primers may be used to perform PCR amplification of a cDNA of

interest from either a cDNA library or a population of cDNAs. As noted above, the appropriate nucleotide sequence for use in a PCR-based method may be obtained from SEQ ID NO:1 either for the purpose of isolating overlapping 5′ and 3′ RACE products for generation of a full-length sequence coding for NHL, or to isolate a portion of the nucleotide sequence coding for NHL for use as a probe to screen one or more cDNA- or genomic-based libraries to isolate a full-length sequence encoding NHL or NHL-like proteins.

This invention also includes vectors containing a NHL gene, host cells containing the vectors, and methods of making substantially pure NHL protein comprising the steps of introducing the NHL gene into a host cell, and cultivating the host cell under appropriate conditions such that NHL is produced. The NHL so produced may be harvested from the host cells in conventional ways. Therefore, the present invention also relates to methods of expressing the NHL protein and biological equivalents disclosed herein, assays employing these gene products, recombinant host cells which comprise DNA constructs which express these proteins, and compounds identified through these assays which act as agonists or antagonists of NHL activity.

The cloned NHL cDNA obtained through the methods described above may be recombinantly expressed by molecular cloning into an expression vector (such as pcDNA3.neo, pcDNA3.1, pCR2.1, pBlueBacHis2 or pLITMUS28) containing a suitable promoter and other appropriate transcription regulatory elements, and transferred into prokaryotic or eukaryotic host cells to produce recombinant NHL. Expression vectors are defined herein as DNA sequences that are required for the transcription of cloned DNA and the translation of their mRNAs in an appropriate host. Such vectors can be used to express eukaryotic DNA in a variety of hosts such as bacteria, blue green algae, plant cells, insect cells and animal cells. Specifically designed vectors allow the shuttling of DNA between hosts such as bacteria-yeast or bacteria-animal cells. An appropriately constructed expression vector should contain: an origin of replication for autonomous replication in host cells, selectable markers, a limited number of useful restriction enzyme sites, a potential for high copy number, and active promoters. A promoter is defined as a DNA sequence that directs RNA polymerase to bind to DNA and initiate RNA synthesis. A strong promoter is one which causes mRNAs to be initiated at high frequency. To determine the NHL cDNA sequence(s) that yields optimal levels of NHL, cDNA molecules including but not

limited to the following can be constructed: a cDNA fragment containing the full-length open reading frame for NHL as well as various constructs containing portions of the cDNA encoding only specific domains of the protein or rearranged domains of the protein. All constructs can be designed to contain none, all or portions of the 5' and/or 3' untranslated region of a NHL cDNA. The expression levels and activity of NHL can be determined following the introduction, both singly and in combination, of these constructs into appropriate host cells. Following determination of the NHL cDNA cassette yielding optimal expression in transient assays, this NHL cDNA construct is transferred to a variety of expression vectors (including recombinant viruses), including but not limited to those for mammalian cells, plant cells, insect cells, oocytes, bacteria, and yeast cells. Techniques for such manipulations can be found described in Sambrook, et al., *supra*, are well known and available to the artisan of ordinary skill in the art. Therefore, another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding the NHL protein. An expression vector containing DNA encoding a NHL-like protein may be used for expression of NHL in a recombinant host cell. Such recombinant host cells can be cultured under suitable conditions to produce NHL or a biologically equivalent form. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses. Commercially available mammalian expression vectors which may be suitable for recombinant NHL expression, include but are not limited to, pcDNA3.neo (Invitrogen), pcDNA3.1 (Invitrogen), pCI-neo (Promega), pLITMUS28, pLITMUS29, pLITMUS38 and pLITMUS39 (New England Bioloabs), pcDNAI, pcDNAIamp (Invitrogen), pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and lZD35 (ATCC 37565). Also, a variety of bacterial expression vectors may be used to express recombinant NHL in bacterial cells. Commercially available bacterial expression vectors which may be suitable for recombinant NHL expression include, but are not limited to pCR2.1 (Invitrogen), pET11a (Novagen), lambda gt11 (Invitrogen), and pKK223-3 (Pharmacia). In addition, a variety of fungal cell expression vectors may be used to express recombinant NHL in fungal cells. Commercially available fungal cell expression vectors which may be suitable for

recombinant NHL expression include but are not limited to pYES2 (Invitrogen) and *Pichia* expression vector (Invitrogen). Also, a variety of insect cell expression vectors may be used to express recombinant protein in insect cells. Commercially available insect cell expression vectors which may be suitable for recombinant expression of NHL include but are not limited to pBlueBacIII and pBlueBacHis2 (Invitrogen), and pAcG2T (Pharmingen).

Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of bovine, porcine, monkey and rodent origin; and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. For instance, one insect expression system utilizes *Spodoptera frugiperda* (Sf21) insect cells (Invitrogen) in tandem with a baculovirus expression vector (pAcG2T, Pharmingen). Also, mammalian species which may be suitable and which are commercially available, include but are not limited to, L cells L-M(TK$^-$) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), Saos-2 (ATCC HTB-85), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171) and CPAE (ATCC CCL 209).

As disclosed in Example section 1, a 115 kb BAC clone (from Genome Systems) was subcloned and subjected to restriction and sequence analysis. Four genes at chromosome location 20q13.3 were identified, including M68/DcR3, NHL, SCLIP and ARP (Figure 5A). The nucleotide sequence of this BAC clone, hbm168, is presented as follows:

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TGAAGAGCTT TGACCAAGAG GCTGTGACGA GGCCCTACGA GGACTCTGGC TCTCCTCCTG     60
CTAAGCACAC CCAGGCAGGT GTCCTGGCAG ATGAGGACCA CATGCAGAGC CTCGGCCAGC    120
CCACCAATGC CCGGATATGC AAGTGAGCCC AGCCTGGACC CCCCGGCGAG GCCCAGCAGC    180
ACCAGCCCAG GCCCGAAAAC CTTAAGAAAT GACCAGTGTC TGCTGCTTTA AGCCACCAAG    240
CTCTGCGGTG GTTTGTTAGG CTGCAAGCAT GGCTAATTCA GAAACTGCCA GAAACAAGCA    300
CTGCTGTCCC CAGCCTGGGA CACACAGCAC CGCCTCTGCG TGGGGAGAGG GCACAGGCTA    360
AGGGCACAAA TGCCATCCCA GACCCGGCTC TTGTGTGTGG AAGGGGCCAC TGTGCCATGA    420
GGCAGAGGAA ACCTTGGCAG GACCTTATGC CACAGCAATT TAAAAGAGAA GAAACAGGCT    480
GGGCGTGGTG GCTCATGCCT ATAATCCCAG CACTTTGGGA GGCCAAGGTG GTGGATCACT    540
TGAGGTCAGG AGTTCAAGAC CAGCCTGGCC AATATGGTGA AACCCTGTCT CTACGAAAAA    600
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TACAAAATTT AGGCAGGCGT GGTGGCGGGT GCCTGTAATC CCTGCTATTC AGGAGGCTGA 660
GGCAAGAGAT TTACTTGAAC CCAGGAGGTG GAGGCTGCTG CAGTGAGCTG AGATCATGCC 720
ACTGCACTCC AGCCTGTGTG ACGGAGTGAG ACTTGGTCTC AAAAAAAAAA AAGGAAACAC 780
ATCTGACTAG TGTGATCTCG CAAGGAACAT TCCAGACACA GTGGAGCTAG AAGGTTCTTC 840
TCCAAACAAG GAATCCCCAG GGGATCAAAT TGTTTTGCAT CGGCCAGACA TGGTGGCTCA 900
AGCCTGTAAC CCCAGTGCTT CGGGAGGCTG AGGTGGGAGG ACTGCTTGAG TCCAGGAGTT 960
CAAGACTAGC TTGGGCAACA CAGTGAGAGC CCATTAGCCA GGCGTGGTGG CACATGCCTG 1020
CAGTCCCAGC ACTGTACTAA AAATCTACAC GGGGCCGGGC ATGGTGGCAC ATGCCTGTAG 1080
AGTCCCAGCT ACTCAGGAGG CTGAGGCAGG ACGATTCCTT GAACCCAGGA GGTCACGGCT 1140
GCCATGAGCC GTGACTGTGC CACTGCACTC CAGTCTGTGC AACAGAACGA GACTCTGTTT 1200
CGAAAAACAA AAAATCATTT CATGTCTCCA GTTTCTCCAC TGGCAAAAGA CTCTGTCAAG 1260
GTAAAAAATG GTTCTGACCC ACAGAAATCT AAGAAAGGAA AAAATATAAA AAATAGAAAA 1320
TTTAAAAAAG AGATGGTCTC AGAATAAAGA CCAACCTGGG CTATGGTTGT CACTCTTCCC 1380
TCACACCTTA GAAAGCTTTC TGGCCGCATC TGGCCAAAGG GCCACCCTGC CCCATCTTGG 1440
ATCAGTGAGG TGCCTTCGAA CAAGCCACCT GCCCTGGAGC CCGTCCTGTC TTGTCTGCCA 1500
CCGCACGCTC AGTAGGGGAG GGGAAGTCGC TAGGTTTTAG TTCACCAGTC TCTGGATCAA 1560
GACGTGCCAT AACCAAGAAG CCCCAGCCAC ACCCAGACCC GATGTGGCCA CAAGGGGTGA 1620
GCTGGGAAGG CCCAGGAAAA GGCGGGAGGC GGACGAATGG AAATGTCATT CTGTGGCCAC 1680
AGAAATGATC TCAACGTTTT GTAACTTCCT ACCAAGAGGC AGTCTTAGCT CTGCCCTTGA 1740
ACCAGCACTT GGTGATGTCG CTTGCGTCAA TCAAGGCAAC AGAAGTGAGC AGGAGGCCCA 1800
CTTTCCTCTG CAACTGTGGG CTTACGGGGC AAAGAAGTCC AGGCCTCCAG GTGGAGGATC 1860
ACAGACCGGG CAAAGCAGAG GAGAGCCACC CAGCCGAGCC TACCTGTGCC TCAGACTGCC 1920
TCCCTCCAGA GACCCCTGTG GCCAAGGCCA CCCAGACCAG CAGGTCCTTG CCAAGCTGTC 1980
AGCTGACGAC AGGGGTTGGT GAGGCCGGCC CAGACCAGCA GAACCACGAA CCAACCAACA 2040
GAATTAAAAA TAATAACAAC TATGTCTTGT CTTAAGCCAC TAAGTTTTGG ATGGTTTCTT 2100
TCTTTCTTTT TCTTTTTTTT TTTCGGAGAC GCAGTCTCAC TCTGTTGCCC AGGCTGGAGT 2160
GCAGTGGCGC AATCTTGGCT CACTGCAAGC TCTGCCCCCC GGATTCACGC CATTCCCCTG 2220
CCTCAGCCTC CTGAGTAACT GGGACTACAG GTGCCTGCCA TTGGGTGTTT TCTTAAACAG 2280
CAAAAGAAAA CTGACACAAT CATAAACAGA GCAAGCAAGA GAACTTGGCA ATTATTTCCT 2340
CTCTACTTCT CACTGTTCTT CAAAGAGTTA ACTCAAGCAT AAGATGTGAG CAAATTCTTT 2400
TAACATCCTA GAAAAAAAGC TCCTACTCAG TGTTCATAAA GCAAAGCTAA CCTACAGGAG 2460
CCACCTTCCA CAGTGACCAC AGGAAACCAA GACAGCAAGT GGGACACCAG CCTCCAGGGC 2520
ACTGCGCCAG CCGTGCGCCT GTGTCTGCCA CTGCCCTGGT CCGTCACTGC CACCAGCCGG 2580
CAAGACACCC ACAGAGGAGA GCTCTAAGCC ACAACTGTGT ACGAAGACAA CTGTGCAGGA 2640

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TTTTATTACT ACAACATTTT TGTTTTCTTT TTTTTTTTTT TTTGAGACTG AGTCTCGCTC 2700
TGTCACCCAG GCTGGAGTGC AGTGGCACAA TCTCGGCTCA CTGTAACCTC CATCTCCCTG 2760
GTTCAAGCAA TTCTCCTGCT GCAGCCTCCC AACTGGATTA CAGGCGCCCG CCACCACGCC 2820
TGGCTAATTT TTGTACTTTT AGTAGAGATG GGGTTTCACC ATGTTGGCCA GACTGGTCTC 2880
AAATTCCTGA CAAGTGATCC ACCCACCCTG GCCTCCCAAA GTGCTGGGAT TACAGGTGTG 2940
AGCCACTGCG CCTGGCCCAT TTTTGTTTAT CAATAAAAAT GTACTTAATG TTGAACTCTC 3000
CACATTTCAA ATGGGTAACT CCAGTGTCCT TGATGCTCCT GCGACATGTT CGTGAGACTT 3060
CTCTTGGGTG TGAGAGTCTA GCATGTGGGT GGTCTGGACA GGAGGGGGAG GGAAGAGTGC 3120
AGAGCCGGGC AGGGTAAAGA GACCCCCTAG GATGTGAAGG CCGCCCTGCA TTTGTCAGAC 3180
TGGGCAACAC CCACTCCATC AGATGGACCC TGGTATGGGC GGCAAGCCAC CTAGGTGCCG 3240
AGGCAAGAGA CCGAGGGCAC GAGCTGTTCC GGTGTAATAA AATGCATAAA ATAAGAATAG 3300
TTATACTAGA TATAGATCAT AAATATGATT ATATATGAAT ATCATTCATC ATTAGTTTGT 3360
AGCAATTACT CTTTATTCCA ATATTATAAT AATCCTTGCC TAAGCATAAC CTAGGAAAAA 3420
CTAGGAAATC ATAACCTAGG AAAAACTAGG CCATACAGAG ATAGGAGCTG AGGGGACATA 3480
GTGAGAACTG ACCAGAAGAC AAGAGTGCGA GCCTTCTGTT ATGCCTGGAC AGGGCCACCA 3540
GAGGGCTCCT TGGTCTAGCG GTAACGCCAG CATCTGGGAA GACGCCCGTT GCCAAGTGGA 3600
CCGTGGTCTA GCGGTAGCCT CAGTGTCAAG GAAAAACACC CGCTACTTAG CAAACCAGGA 3660
AAGAGAGTCT CCCTTTCCCC GGGGGAGTTT AGAGAAGACT CTACTCCTCC ACCTCTTGCG 3720
GAGGGCCTGA CATCAGTCAG GCCCGCCCGC AGTTATCCGG AGGCCTAACC GTCTCCCTGT 3780
GATGCTGTGC TTCAGTGGTC ACGCTCCTAG TCCGCCTTCA TGTTCCATCC TGTGCACCTG 3840
GCTCTGCCTT CTAGATAGCA GCAGCAAATT AGTGAAAGTA CTGAAAGTCT CTGATAAGCA 3900
GAAATAATGG CGTAAGCGGT CTCTCTCTCT CTCTCCTCTC TCTCTGCCTC AGCTGCCAGG 3960
AAGGGAAGGG CCCCCTGGCC AGTGGGCACG TGACCCACAT GACCTTACCT ATCACTGGAC 4020
ATGGTTCACA CTCCTTACCC TGCCGCTTTG TCTTGTATCC AATAAATAGC GCAACCTGGC 4080
ATTCGGGGCC GCTACCAGTC TCCGCGTCTT GGTGGTAGTG GTCCCCCAGG CCCAGCTGTC 4140
TTTTTCTTTT ATCTTTGTCT TGTGTCTTTA TTTCTACACT CTCTCATCTC CGCATACGAG 4200
GAGAAAACCC ACCAACCCTG TGGGGCTGGT CCCTACACCC TGGCTTTGTA GACTGGAGCC 4260
TAGGCACGAC TCAGCTGCTG TAGTGAATTG CGATCCTCCA AACCCAGCAA GGCACCTGCA 4320
GGACATCTGG CCCAGTCTCC TCGTTGAGCC AGTTCACGAA AAAGAGACTT TTCTGAGTGA 4380
CATGCTAATG GGCAATATGA GGACTAAATG GGATGGTCTC CAACTTGGAC AAACCAACAG 4440
TAAAAGCCAC TTTGCGGGGA AAGAAACTTT TCCTTTTTTC TTTTTTTTGA GACAGGATCT 4500
CACCCTGTCA CCCAGGCTGC AGTGCAGTGG CATGACCTTG GCTCACTGCA GCCTCAACCT 4560
CTCTCAGGCT CAAGCAATCC TCCCGCCTCA ACCTCCCATG CAGCTGGGAC CATAGGTGCA 4620
TGCCACCACA CCCAAATAAT TTTTATATTT TTTGTAGAGA CGAGGTTTCA CTATGTTGCT 4680
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CGGGCTGGTC TCAACTCCTG GGCTCAAGCA ACCCTCCCAC CTCAGCCTCC CAAAGTGCTC 4740
AGATTACAGG CAGGAGCCAC CAGGCCTGGC CAACATAGGA AGAAATTTAA ATTTGAATTG 4800
AATATTAGAA GAGATGAAAA TTCATCAACA TGGAAAGACA AAGATCATTA ACTAAAGCCA 4860
AACCAGAATG GAAGCTGTGT GTACAGTGGG GTCTCATGCT GGGAACGCGA GGGGCACGTG 4920
CAGGGCTCCA CGGTGTGGCG ACGCCCCATG CTCCCTTTGT GGGGGTTCAT CCAGCGGAAC 4980
ATGAGGACCT GGGGTGCTTT TCAACATGTA CGTGAGTTTA ATAATAAAAA GGTTTAAGGA 5040
AAGAAAAATT CATATGTTTC TATATAAACA GAACATCTGG AAAGATCTAT TCTAAGGTGT 5100
TGACAGTAGG AATCTCTAGG TAGTAGTAAT ATGGCCTTTT TGAATTTTTG CTTATCAGTA 5160
TTTTCTAATT TTCTTTTTCT TTCTAAATAA TTCTAGCTAT GAAATAATTT TCTACCATAT 5220
ATATTTTGTA ATAAAAATGG TTATATTTAA TTTTTTAAAG GCTGTACAAA CTTCCTGATA 5280
AAATGGCAAA TTAGACACAC ACATGTGGGC CGGGTACAGT GGCTCGCGCC TGTAATTCCA 5340
GCACTTTGGG AGGCTGAGGC AGGCAGATCA CCTAAGGTCA GGAGTTTGAG ACCAGCCTGG 5400
CCAACATGGT GAAACCCCGT CTCTACTAAA TATACAAAAA TGAGCTGGAT GTGGTGGCAC 5460
ACACCTATAG TGCCAGCTAC TTGGGAAGCT GAGGCAGGAA AATTGCTTCA ACCCGGGAGG 5520
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CAAATTTTTT TCCTGCCCCT CTCTCATCTC CTTTTGGGAG TACCACTGCA TGTATGTTGG 114780
TGTGCGTTCT CTA (SEQ ID NO:3)                                      114793.
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The present invention also relates to a portion of SEQ ID NO:3 which comprises 5' regulatory regions, exons, introns and 3' non-translated regions which comprise the human NHL gene of the present invention. Such regulatory sequence may be found within the various regions of this 115 kb fragment. The 5' portion of SEQ ID NO:1 begins at nucleotide 47095 of SEQ ID NO:3, the initiating ATG of human NHL is from nucleotide 48687-48689 of SEQ ID NO:3, the termination 'TAG' codon is from nucleotide 84855-84857, while the 3' terminus of SEQ ID NO:1 as disclosed herein (GCAGTGCCC) corresponds to nucleotides 85308-85316. To this end, one preferred aspect of the invention is an isolated genomic fragment or fragments which comprise from about nucleotide 470000 to about nucleotide 85500 of SEQ ID NO:3), which comprises the portion of the genomic clone encoding the mRNA transcript responsible for human NHL (see Figure 5A-B). The genomic sequence encoding NHL contains 35 exons (Figure 5A). An especially preferred aspect of the invention is a human genomic fragment or fragments which comprise from about nucleotide 47095 to about nucleotide 85316 of SEQ ID NO:3. As noted in regard to SEQ ID NO:1, the present invention also relates to DNA vectors and recombinant hosts which comprise at least a portion of SEQ ID NO:3. Portions of the 115 kb genomic fragment may be housed in multiple vector/hosts so as to optimize handling of the DNA sequences within SEQ ID NO:3. Therefore, the present invention relates to the isolated genomic sequence which set forth as SEQ ID NO:3, a region of SEQ ID NO:3 which contains the coding and non-coding region of human NHL, as well as *cis*-acting sequences within SEQ ID NO:3 which effect regulation of transcription of one or more of the genes localized within this 115 kb human genomic fragment, including regulatory regions effecting levels of NHL, M68/DcR3, SCLIP and ARP. As noted above, this region of chromosome 20 (20q13.3) is associated with tumor growth. Therefore, an aspect of this invention also comprises, as one example, the use of one or more regulatory regions of this 115 kb genomic sequence as a target to antagonize the effect of a transcriptional factor(s) which normally upregulate expression of a gene which has a caustic role in tumor growth. Alternatively, compounds may be selected which interacts with a specific *cis*-acting sequence to upregulate a gene within this region, where upregulation results in a decrease in tumor growth.

The present invention is also directed to methods of screening for compounds

which modulate the expression of DNA or RNA encoding a NHL protein. Compounds which modulate these activities may be DNA, RNA, peptides, proteins, or non-proteinaceous organic molecules. Compounds may modulate by increasing or attenuating the expression of DNA or RNA encoding NHL, or the function of the NHL-based protein. Compounds that modulate the expression of DNA or RNA encoding NHL or the biological function thereof may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there is a change in expression or function. The assay may be made quantitative by comparing the expression or function of a test sample with the levels of expression or function in a standard sample. Kits containing NHL, antibodies to NHL, or modified NHL may be prepared by known methods for such uses.

The DNA molecules, RNA molecules, recombinant protein and antibodies of the present invention may be used to screen and measure levels of NHL. The recombinant proteins, DNA molecules, RNA molecules and antibodies lend themselves to the formulation of kits suitable for the detection and typing of NHL. Such a kit would comprise a compartmentalized carrier suitable to hold in close confinement at least one container. The carrier would further comprise reagents such as recombinant NHL or anti-NHL antibodies suitable for detecting NHL. The carrier may also contain a means for detection such as labeled antigen or enzyme substrates or the like.

The assays described above can be carried out with cells that have been transiently or stably transfected with NHL. The expression vector may be introduced into host cells via any one of a number of techniques including but not limited to transformation, transfection, protoplast fusion, and electroporation. Transfection is meant to include any method known in the art for introducing NHL into the test cells. For example, transfection includes calcium phosphate or calcium chloride mediated transfection, lipofection, infection with a retroviral construct containing NHL, and electroporation. The expression vector-containing cells are individually analyzed to determine whether they produce NHL protein. Identification of NHL expressing cells may be done by several means, including but not limited to immunological reactivity with anti-NHL antibodies, labeled ligand binding, the presence of host cell-associated NHL activity.

The specificity of binding of compounds showing affinity for NHL is shown by measuring the affinity of the compounds for recombinant cells expressing NHL.

Expression of human NHL and screening for compounds that bind to NHL or that inhibit the binding of a known, radiolabeled ligand of NHL provides an effective method for the rapid selection of compounds with high affinity for NHL. Such ligands need not necessarily be radiolabeled but can also be nonisotopic compounds that can be used to displace bound radiolabeled compounds or that can be used as activators in functional assays. Compounds identified by the above method are likely to be agonists or antagonists of NHL and may be peptides, proteins, or non-proteinaceous organic molecules.

Accordingly, the present invention is directed to methods for screening for compounds which modulate the expression of DNA or RNA encoding a NHL protein as well as compounds which effect the function of the NHL protein. Methods for identifying agonists and antagonists of other receptors are well known in the art and can be adapted to identify agonists and antagonists of NHL. For example, Cascieri et al. (1992, *Molec. Pharmacol.* 41:1096-1099) describe a method for identifying substances that inhibit agonist binding to rat neurokinin receptors and thus are potential agonists or antagonists of neurokinin receptors. The method involves transfecting COS cells with expression vectors containing rat neurokinin receptors, allowing the transfected cells to grow for a time sufficient to allow the neurokinin receptors to be expressed, harvesting the transfected cells and resuspending the cells in assay buffer containing a known radioactively labeled agonist of the neurokinin receptors either in the presence or the absence of the substance, and then measuring the binding of the radioactively labeled known agonist of the neurokinin receptor to the neurokinin receptor. If the amount of binding of the known agonist is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of the neurokinin receptor. Where binding of the substance such as an agonist or antagonist to is measured, such binding can be measured by employing a labeled substance or agonist. The substance or agonist can be labeled in any convenient manner known to the art, e.g., radioactively, fluorescently, enzymatically.

Therefore, the present invention includes assays by which modulators of NHL are identified. As noted above, methods for identifying agonists and antagonists are known in the art and can be adapted to identify compounds which effect *in vivo* levels of NHL. Accordingly, the present invention includes a method for determining whether a substance is a potential modulator of mammalian NHL levels that

comprises:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of NHL in the cells;

(b) exposing the test cells to the substance;

(c) measuring the amount of binding of the substance to NHL;

(d) comparing the amount of binding of the substance to NHL in the test cells with the amount of binding of the substance to control cells that have not been transfected with NHL or a portion thereof; wherein if the amount of binding of the substance is greater in the test cells as compared to the control cells, the substance is capable of binding to NHL.

The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: e.g., physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

The assays described above can be carried out with cells that have been transiently or stably transfected with NHL. Transfection is meant to include any method known in the art for introducing NHL into the test cells. For example, transfection includes calcium phosphate or calcium chloride mediated transfection, lipofection, infection with a retroviral construct containing NHL, and electroporation.

Where binding of the substance or agonist to NHL is measured, such binding can be measured by employing a labeled substance or agonist. The substance or agonist can be labeled in any convenient manner known to the art, e.g., radioactively, fluorescently, enzymatically.

Therefore, the specificity of binding of compounds having affinity for NHL shown by measuring the affinity of the compounds for recombinant cells expressing the cloned receptor or for membranes from these cells. Expression of the cloned receptor and screening for compounds that bind to NHL or that inhibit the binding of a known, radiolabeled ligand of NHL to these cells provides an effective method for the rapid selection of compounds with high affinity for NHL. Such ligands need not necessarily be radiolabeled but can also be nonisotopic compounds that can be used to displace bound radiolabeled compounds or that can be used as activators in functional assays. It is also possible to construct assays wherein compounds are tested for an ability to modulate helicase activity in an *in vitro-* or *in vivo-* based assay. Compounds identified by the above method again are likely to be agonists or

antagonists of NHL and may be peptides, proteins, or non-proteinaceous organic molecules. As noted elsewhere in this specification, compounds may modulate by increasing or attenuating the expression of DNA or RNA encoding NHL, or by acting as an agonist or antagonist of the NHL receptor protein. Again, these compounds that modulate the expression of DNA or RNA encoding NHL or the biological function thereof may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there is a change in expression or function. The assay may be made quantitative by comparing the expression or function of a test sample with the levels of expression or function in a standard sample.

Expression of NHL DNA may also be performed using *in vitro* produced synthetic mRNA. Synthetic mRNA can be efficiently translated in various cell-free systems, including but not limited to wheat germ extracts and reticulocyte extracts, as well as efficiently translated in cell based systems, including but not limited to microinjection into frog oocytes, with microinjection into frog oocytes being preferred.

Following expression of NHL in a host cell, NHL protein may be recovered to provide NHL protein in active form. Several NHL protein purification procedures are available and suitable for use. Recombinant NHL protein may be purified from cell lysates and extracts by various combinations of, or individual application of salt fractionation, ion exchange chromatography, size exclusion chromatography, hydroxylapatite adsorption chromatography and hydrophobic interaction chromatography. In addition, recombinant NHL protein can be separated from other cellular proteins by use of an immunoaffinity column made with monoclonal or polyclonal antibodies specific for full-length NHL protein, or polypeptide fragments of NHL protein.

Polyclonal or monoclonal antibodies may be raised against NHL or a synthetic peptide (usually from about 9 to about 25 amino acids in length) from a portion of NHL disclosed in SEQ ID NO:2. Monospecific antibodies to NHL are purified from mammalian antisera containing antibodies reactive against NHL or are prepared as monoclonal antibodies reactive with NHL using the technique of Kohler and Milstein (1975, *Nature* 256: 495-497). Monospecific antibody as used herein is defined as a single antibody species or multiple antibody species with homogenous binding characteristics for NHL. Homogenous binding as used herein refers to the ability of the antibody species to bind to a specific antigen or epitope, such as those associated

with NHL, as described above. Human NHL-specific antibodies are raised by immunizing animals such as mice, rats, guinea pigs, rabbits, goats, horses and the like, with an appropriate concentration of NHL protein or a synthetic peptide generated from a portion of NHL with or without an immune adjuvant.

Preimmune serum is collected prior to the first immunization. Each animal receives between about 0.1 mg and about 1000 mg of NHL protein associated with an acceptable immune adjuvant. Such acceptable adjuvants include, but are not limited to, Freund's complete, Freund's incomplete, alum-precipitate, water in oil emulsion containing *Corynebacterium parvum* and tRNA. The initial immunization consists of NHL protein or peptide fragment thereof in, preferably, Freund's complete adjuvant at multiple sites either subcutaneously (SC), intraperitoneally (IP) or both. Each animal is bled at regular intervals, preferably weekly, to determine antibody titer. The animals may or may not receive booster injections following the initial immunization. Those animals receiving booster injections are generally given an equal amount of NHL in Freund's incomplete adjuvant by the same route. Booster injections are given at about three week intervals until maximal titers are obtained. At about 7 days after each booster immunization or about weekly after a single immunization, the animals are bled, the serum collected, and aliquots are stored at about -20°C.

Monoclonal antibodies (mAb) reactive with NHL are prepared by immunizing inbred mice, preferably Balb/c, with NHL protein. The mice are immunized by the IP or SC route with about 1 mg to about 100 mg, preferably about 10 mg, of NHL protein in about 0.5 ml buffer or saline incorporated in an equal volume of an acceptable adjuvant, as discussed above. Freund's complete adjuvant is preferred. The mice receive an initial immunization on day 0 and are rested for about 3 to about 30 weeks. Immunized mice are given one or more booster immunizations of about 1 to about 100 mg of NHL in a buffer solution such as phosphate buffered saline by the intravenous (IV) route. Lymphocytes, from antibody positive mice, preferably splenic lymphocytes, are obtained by removing spleens from immunized mice by standard procedures known in the art. Hybridoma cells are produced by mixing the splenic lymphocytes with an appropriate fusion partner, preferably myeloma cells, under conditions which will allow the formation of stable hybridomas. Fusion partners may include, but are not limited to: mouse myelomas P3/NS1/Ag 4-1; MPC-11; S-194 and Sp 2/0, with Sp 2/0 being preferred. The antibody producing cells and myeloma cells are fused in polyethylene glycol, about 1000 mol. wt., at concentrations from about

30% to about 50%. Fused hybridoma cells are selected by growth in hypoxanthine, thymidine and aminopterin supplemented Dulbecco's Modified Eagles Medium (DMEM) by procedures known in the art. Supernatant fluids are collected form growth positive wells on about days 14, 18, and 21 and are screened for antibody production by an immunoassay such as solid phase immunoradioassay (SPIRA) using NHL as the antigen. The culture fluids are also tested in the Ouchterlony precipitation assay to determine the isotype of the mAb. Hybridoma cells from antibody positive wells are cloned by a technique such as the soft agar technique of MacPherson, 1973, Soft Agar Techniques, in *Tissue Culture Methods and Applications*, Kruse and Paterson, Eds., Academic Press.

Monoclonal antibodies are produced *in vivo* by injection of pristine primed Balb/c mice, approximately 0.5 ml per mouse, with about 2 x 10^6 to about 6 x 10^6 hybridoma cells about 4 days after priming. Ascites fluid is collected at approximately 8-12 days after cell transfer and the monoclonal antibodies are purified by techniques known in the art.

In vitro production of anti- NHL mAb is carried out by growing the hybridoma in DMEM containing about 2% fetal calf serum to obtain sufficient quantities of the specific mAb. The mAb are purified by techniques known in the art.

Antibody titers of ascites or hybridoma culture fluids are determined by various serological or immunological assays which include, but are not limited to, precipitation, passive agglutination, enzyme-linked immunosorbent antibody (ELISA) technique and radioimmunoassay (RIA) techniques. Similar assays are used to detect the presence of NHL in body fluids or tissue and cell extracts.

It is readily apparent to those skilled in the art that the above described methods for producing monospecific antibodies may be utilized to produce antibodies specific for NHL peptide fragments, or a respective full-length NHL.

NHL antibody affinity columns are made, for example, by adding the antibodies to Affigel-10 (Biorad), a gel support which is pre-activated with N-hydroxysuccinimide esters such that the antibodies form covalent linkages with the agarose gel bead support. The antibodies are then coupled to the gel via amide bonds with the spacer arm. The remaining activated esters are then quenched with 1M ethanolamine HCl (pH 8). The column is washed with water followed by 0.23 M glycine HCl (pH 2.6) to remove any non-conjugated antibody or extraneous protein. The column is then equilibrated in phosphate buffered saline (pH 7.3) and the cell

culture supernatants or cell extracts containing full-length NHL or NHL protein fragments are slowly passed through the column. The column is then washed with phosphate buffered saline until the optical density (A_{280}) falls to background, then the protein is eluted with 0.23 M glycine-HCl (pH 2.6). The purified NHL protein is then dialyzed against phosphate buffered saline.

Pharmaceutically useful compositions comprising modulators of NHL may be formulated according to known methods such as by the admixture of a pharmaceutically acceptable carrier. Examples of such carriers and methods of formulation may be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the protein, DNA, RNA, modified NHL, or either NHL agonists or antagonists including tyrosine kinase activators or inhibitors.

Therapeutic or diagnostic compositions of the invention are administered to an individual in amounts sufficient to treat or diagnose disorders. The effective amount may vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration.

The pharmaceutical compositions may be provided to the individual by a variety of routes such as subcutaneous, topical, oral and intramuscular.

The term "chemical derivative" describes a molecule that contains additional chemical moieties which are not normally a part of the base molecule. Such moieties may improve the solubility, half-life, absorption, etc. of the base molecule. Alternatively the moieties may attenuate undesirable side effects of the base molecule or decrease the toxicity of the base molecule. Examples of such moieties are described in a variety of texts, such as Remington's Pharmaceutical Sciences.

Compounds identified according to the methods disclosed herein may be used alone at appropriate dosages. Alternatively, co-administration or sequential administration of other agents may be desirable.

The present invention also has the objective of providing suitable topical, oral, systemic and parenteral pharmaceutical formulations for use in the novel methods of treatment of the present invention. The compositions containing compounds identified according to this invention as the active ingredient can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compounds can be administered in such oral dosage forms as tablets,

capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they may also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts.

Advantageously, compounds of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily. Furthermore, compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

For combination treatment with more than one active agent, where the active agents are in separate dosage formulations, the active agents can be administered concurrently, or they each can be administered at separately staggered times.

The dosage regimen utilizing the compounds of the present invention is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal, hepatic and cardiovascular function of the patient; and the particular compound thereof employed. A physician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentrations of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug.

The present invention also relates to a non-human transgenic animal which is useful for studying the ability of a variety of compounds to act as modulators of NHL, or any alternative functional NHL *in vivo* by providing cells for culture, *in vitro*. In reference to the transgenic animals of this invention, reference is made to transgenes and genes. As used herein, a transgene is a genetic construct including a gene. The transgene is integrated into one or more chromosomes in the cells in an animal by

methods known in the art. Once integrated, the transgene is carried in at least one place in the chromosomes of a transgenic animal. Of course, a gene is a nucleotide sequence that encodes a protein, such as one or a combination of the cDNA clones described herein. The gene and/or transgene may also include genetic regulatory elements and/or structural elements known in the art. A type of target cell for transgene introduction is the embryonic stem cell (ES). ES cells can be obtained from pre-implantation embryos cultured *in vitro* and fused with embryos (Evans et al., 1981, *Nature* 292:154-156; Bradley et al., 1984, *Nature* 309:255-258; Gossler et al., 1986, *Proc. Natl. Acad. Sci.* USA 83:9065-9069; and Robertson et al., 1986 *Nature* 322:445-448). Transgenes can be efficiently introduced into the ES cells by a variety of standard techniques such as DNA transfection, microinjection, or by retrovirus-mediated transduction. The resultant transformed ES cells can thereafter be combined with blastocysts from a non-human animal. The introduced ES cells thereafter colonize the embryo and contribute to the germ line of the resulting chimeric animal (Jaenisch, 1988, *Science* 240: 1468-1474). It will also be within the purview of the skilled artisan to produce transgenic or knock-out invertebrate animals (e.g., *C. elegans*) which express the NHL transgene in a wild type background as well in *C. elegans* mutants knocked out for one or both of the NHL subunits. These organisms will be helpful in further determining the dominant negative effect of NHL as well as selecting from compounds which modulate this effect.

The present invention also relates to a non-human transgenic animal which is heterozygous for a functional NHL gene native to that animal. As used herein, functional is used to describe a gene or protein that, when present in a cell or *in vitro* system, performs normally as if in a native or unaltered condition or environment. The animal of this aspect of the invention is useful for the study of the retinal specific expression or activity of NHL in an animal having only one functional copy of the gene. The animal is also useful for studying the ability of a variety of compounds to act as modulators of NHL activity or expression *in vivo* or, by providing cells for culture, *in vitro*. It is reiterated that as used herein, a modulator is a compound that causes a change in the expression or activity of NHL, or causes a change in the effect of the interaction of NHL with its ligand(s), or other protein(s). In an embodiment of this aspect, the animal is used in a method for the preparation of a further animal which lacks a functional native NHL gene. In another embodiment, the animal of this aspect is used in a method to prepare an animal which expresses a non-native NHL

gene in the absence of the expression of a native NHL gene. In particular embodiments the non-human animal is a mouse. In further embodiments the non-native NHL is a wild-type human NHL which is disclosed herein, or any other biologically equivalent form of human NHL gene as also disclosed herein.

In reference to the transgenic animals of this invention, reference is made to transgenes and genes. As used herein, a transgene is a genetic construct including a gene. The transgene is integrated into one or more chromosomes in the cells in an animal by methods known in the art. Once integrated, the transgene is carried in at least one place in the chromosomes of a transgenic animal. Of course, a gene is a nucleotide sequence that encodes a protein, such as human or mouse NHL. The gene and/or transgene may also include genetic regulatory elements and/or structural elements known in the art.

Another aspect of the invention is a non-human animal embryo deficient for native NHL expression. This embryo is useful in studying the effects of the lack of NHL on the developing animal. In particular embodiments the animal is a mouse. The animal embryo is also useful as a source of cells lacking a functional native NHL gene. The cells are useful in *in vitro* culture studies in the absence of NHL.

An aspect of this invention is a method to obtain an animal in which the cells lack a functional gene NHL native to the animal. The method includes providing a gene for an altered form of the NHL gene native to the animal in the form of a transgene and targeting the transgene into a chromosome of the animal at the place of the native NHL gene. The transgene can be introduced into the embryonic stem cells by a variety of methods known in the art, including electroporation, microinjection, and lipofection. Cells carrying the transgene can then be injected into blastocysts which are then implanted into pseudopregnant animals. In alternate embodiments, the transgene-targeted embryonic stem cells can be coincubated with fertilized eggs or morulae followed by implantation into females. After gestation, the animals obtained are chimeric founder transgenic animals. The founder animals can be used in further embodiments to cross with wild-type animals to produce F1 animals heterozygous for the altered NHL gene. In further embodiments, these heterozygous animals can be interbred to obtain the non-viable transgenic embryos whose somatic and germ cells are homozygous for the altered NHL gene and thereby lack a functional NHL gene. In other embodiments, the heterozygous animals can be used to produce cells lines. In preferred embodiments, the animals are mice.

A further aspect of the present invention is a transgenic non-human animal which expresses a non-native NHL on a native NHL null background. In particular embodiments, the null background is generated by producing an animal with an altered native NHL gene that is non-functional, *i.e.* a knockout. The animal can be heterozygous (*i.e.*, having a different allelic representation of a gene on each of a pair of chromosomes of a diploid genome) or homozygous (*i.e.*, having the same representation of a gene on each of a pair of chromosomes of a diploid genome) for the altered NHL gene and can be hemizygous (*i.e.*, having a gene represented on only one of a pair of chromosomes of a diploid genome) or homozygous for the non-native NHL gene. In preferred embodiments, the animal is a mouse. In particular embodiments the non-native NHL gene can be a wild-type or mutant allele including those mutant alleles associated with a disease. In further embodiments, the non-native NHL is a human NHL. In a further embodiment the non-native NHL gene is operably linked to a promoter. As used herein, operably linked is used to denote a functional connection between two elements whose orientation relevant to one another can vary. In this particular case, it is understood in the art that a promoter can be operably linked to the coding sequence of a gene to direct the expression of the coding sequence while placed at various distances from the coding sequence in a genetic construct.

An aspect of this invention is a method of producing transgenic animals having a transgene including a non-native NHL gene on a native NHL null background. The method includes providing transgenic animals of this invention whose cells are heterozygous for a native gene encoding a functional NHL protein and an altered native NHL gene. These animals are crossed with transgenic animals of this invention that are hemizygous for a transgene including a non-native NHL gene to obtain animals that are both heterozygous for an altered native NHL gene and hemizygous for a non-native NHL gene. The latter animals are interbred to obtain animals that are homozygous or hemizygous for the non-native NHL and are homozygous for the altered native NHL gene. In particular embodiments, cell lines are produced from any of the animals produced in the steps of the method.

The transgenic animals and cells of this invention are useful in the determination of the *in vivo* function of a non-native NHL in the central nervous system and in other tissues of an animal. The animals are also useful in studying the tissue and temporal specific expression patterns of a non-native NHL throughout the

animals. The animals are also useful in determining the ability for various forms of wild-type and mutant alleles of a non-native NHL to rescue the native NHL null deficiency. The animals are also useful for identifying and studying the ability of a variety of compounds to act as modulators of the expression or activity of a non-native NHL *in vivo*, or by providing cells for culture, for *in vitro* studies.

As used herein, a "targeted gene" or "Knockout" (KO) is a DNA sequence introduced into the germline of a non-human animal by way of human intervention, including but not limited to, the methods described herein. The targeted genes of the invention include nucleic acid sequences which are designed to specifically alter cognate endogenous alleles. An altered NHL gene should not fully encode the same NHL as native to the host animal, and its expression product can be altered to a minor or great degree, or absent altogether. In cases where it is useful to express a non-native NHL gene in a transgenic animal in the absence of a native NHL gene we prefer that the altered NHL gene induce a null lethal knockout phenotype in the animal. However a more modestly modified NHL gene can also be useful and is within the scope of the present invention.

A type of target cell for transgene introduction is the embryonic stem cell (ES). ES cells can be obtained from pre-implantation embryos cultured *in vitro* and fused with embryos (Evans et al., 1981, *Nature* 292:154-156; Bradley et al., 1984, *Nature* 309:255-258; Gossler et al., 1986, *Proc. Natl. Acad. Sci.* USA 83:9065-9069; and Robertson et al., 1986 *Nature* 322:445-448). Transgenes can be efficiently introduced into the ES cells by a variety of standard techniques such as DNA transfection, microinjection, or by retrovirus-mediated transduction. The resultant transformed ES cells can thereafter be combined with blastocysts from a non-human animal. The introduced ES cells thereafter colonize the embryo and contribute to the germ line of the resulting chimeric animal (Jaenisch, 1988, *Science* 240: 1468-1474).

The methods for evaluating the targeted recombination events as well as the resulting knockout mice are readily available and known in the art. Such methods include, but are not limited to DNA (Southern) hybridization to detect the targeted allele, polymerase chain reaction (PCR), polyacrylamide gel electrophoresis (PAGE) and Western blots to detect DNA, RNA and protein.

The following examples are provided to illustrate the present invention without, however, limiting the same hereto.

EXAMPLE 1

Characterization of DNA Molecules Encoding NHL

M68/DcR3 identification - The human osteoprotegerin (OPG) sequence (Acc. # U94332), which is a member of the TNFR-related family, was used to searched Genbank using the programs TBLASTN and TFASTX3 to identify novel gene family members. Two EST sequences (GenBank Acc. # AA155701 and AA025672) were identified that showed sequence similarities to the cysteine repeats of the OPG sequence. These EST sequences were then used to identify additional EST sequences, which formed a single EST cluster (GenBank Acc. #s aa577603, aa603704, aa613366, aa158406, w67560, aa325843, aa155646, aa025673, aa514270, m91489). Two clones were further characterized, which were derived from colon tumor and germ cell tumor libraries (Research Genetics, Inc). DNA sequence analysis revealed two alternatively spliced forms of the 5'-end UTR of M68/DcR3. The M68/DcR3 open reading frame was confirmed by sequence analysis of clones obtained by PCR cloning from a normal human cDNA library (Clontech).

M68/DcR3 BAC identification and sequencing - To further delineate the gene structure of M68/DcR3, genomic DNA was obtained using a human "Down to the Well" ™ genomic bacterial artificial chromosome (BAC) library (Genome Systems, Inc.) according to the manufacturer's protocol. Two sets of PCR primers, C68.36F: 5'-CACAGGTTCAGCATGTTTGTGCGTC-3' (SEQ ID NO:4) and C68.275R: 5'-CACAGTCCCTGCTGGCCTCTGTCTA-3' (SEQ ID NO:5), and E68.715F: 5'-CAGGACATCTCCATCAAGAGGCTGC-3' (SEQ ID NO:6) and E68.972R: 5'-AATAAGAGGGGGCCAGGATCAGTGC-3' (SEQ ID NO:7), were used to carry out PCR reactions to identify positive wells that contained the full-length M68/DcR3 gene. The PCR conditions used were 94°C for 9min, 35 cycles of (94°C, 30 sec., 68°C 3 min.) followed by 72°C for 10 min. Two positive BAC clones were identified and characterized by restriction digestion and BAC-end sequence analyses, of which hbm168 was selected for shotgun sequencing.

A shot-gun library for BAC hbm168 was constructed using a conventional strategy. Briefly, two 150-ml bacterial cultures were combined and purified using a modified protocol of the plasmid-Maxi kit (QIAGEN) followed by CsCl gradient purification. After butanol extraction and isopropanol precipitation, BAC DNA was nebulizied at 10 psi for 60 seconds to generate randomly sheared fragments.

Following ethanol precipitation, the fragments were end-repaired using T4 polymerase (Promega) and BstXI adaptors (Invitrogen) were ligated overnight. Removal of excess, unligated adaptors and size selection was performed using a cDNA sizing column (Life Technologies, Inc.) to generate genomic fragments in the size range of 1500 to 3000 bp. Adaptor ligated fragments were cloned into a modified pBlueScript SK^{+} vector (Stratagene) and transformed in XL2-Blue ultracompentent cells (Stratagene). Approximately 1000 clones were isolated, plasmids were purified using the Turbo miniprep kits (QIAGEN), and both plasmid ends were sequenced with the BigDye terminator kits (Perkin-Elmer). Sequence data were assembled using Phred/Phrap/Consed where single-stranded and gap regions were closed using a directed sequencing strategy.

NHL identification and sequencing – The genomic clone for the NHL gene was obtained and sequenced. The transcript was identified through exon prediction using GRAIL2 and sequence alignment to a contiguous 4.5 kilobase region of chromosome 4 (88% sequence identity). The complete exon structure of NHL was subsequently confirmed by RT-PCR analysis. The exon structure was confirmed by RT-PCR using polyA RNA from a human colorectal adenocarcinoma cell line, SW480 (Clontech). Primers were designed based on the genomic sequence that were predicted to be exons. RT-PCR reaction were carried out with SW480 polyA RNA using standard conditions with TaqGold Enzyme at 94°C for 12min, 35 cycles of (94°C, 30 sec., 60C, 30 sec., and 68°C 2-6 min.) followed by 68°C for 7 min. Most sequence confrimation was accomplished by RT-PCR, although first junction between exon 1 and 2 was confirmed by 5'RACE and junctions between exon 26-29 were by RCCA. The primers used were as follows:

Junction of Exons	Confirmed by Primers
H01/H02	hdkw (5'RACE)
H02/H03	hdiy,hdiz
H03-H09	hdid,hdie,hdja,hdjb
H09-H13	hdja,hdie
H13-H18	hdje,hdjf
H18-H23	hdjg,hdjh
H23-H26	hdji,hdjj
H26-H29	hdkv,r543(RCCA)
H29-H31	hdij,hdmu,hdnd,hdne

H31/H32	hdij,hdmu
H32/H34	hdip,hdil,hdmv,hdik,hdli
H34/H35	hdng,hdnh

HDID - 5'-GTGAATGGCATCCTGGAGAG-3' (SEQ ID NO:8);
HDIE - 5'-GTCTCCAGGCAGCTCAACAG-3' (SEQ ID NO:9);
HDIJ - 5'-ACCCTGTCCCTCCTGTCTGA-3' (SEQ ID NO:10);
HDIY - 5'-AGACCCTAAGATGTTCGGAG-3' (SEQ ID NO:11);
HDIZ - 5'-GATGACCTGTGTGAGTTGCG-3' (SEQ ID NO:12);
HDJA - 5'-CGCAACTCACACAGGTCATC-3' (SEQ ID NO:13);
HDJB - 5'-GGAGTCAGGTCAAAGGATGC-3' (SEQ ID NO:14);
HDJC - 5'-GCATCCTTTGACCTGACTCC-3' (SEQ ID NO:15);
HDJD - 5'-GGTCTGAAACGTGATCTGGG-3' (SEQ ID NO:16);
HDJE - 5'-CCCAGATCACGTTTCAGACC-3' (SEQ ID NO:17);
HDJF - 5'-CGATGATGTGTGGGTTCTCC-3' (SEQ ID NO:18);
HDJG - 5'-GGAGAACCCACACATCATCG-3' (SEQ ID NO:19);
HDJH - 5'-CGTGTCTGAGAAGTCCAGCC-3' (SEQ ID NO:20);
HDJI - 5'-GGCTGGACTTCTCAGACACG-3' (SEQ ID NO:21);
HDJJ - 5'-ACAGCATCTTCTCCACGCAC-3' (SEQ ID NO:22);
HFMU - 5'-AGTCCTCTGGCTTTGCAGTG-3' (SEQ ID NO:23);
HDKV - 5'-TGTGCGTGGAGAAGATGCTG-3' (SEQ ID NO:24);
HDKW - 5'-GGCTGGAAAGGGAAGTCTAC-3' (SEQ ID NO:25);
HDND - 5'-TGGTTCAGGTGCTCTTGGGG-3' (SEQ ID NO:26);
HDNE - 5'-CGTGAAGCAGGAGTTGAGCC-3' (SEQ ID NO:27);
HDIK - 5'-ATCTTGCTCTGGGTCTTCCC-3' (SEQ ID NO:28);
HDIL - 5'-CACTGCAAAGCCAGAGGACT-3' (SEQ ID NO:29);
HDIP - 5'-ATAAGCAAGACGACGACCTC-3' (SEQ ID NO:30);
HDLI - 5'-CTATTCTGTTGGGTGGGTTC-3' (SEQ ID NO:31);
HDMV - 5'-CGTGCCTCCTGTGCTTACCC-3' (SEQ ID NO:32);
HDNG - 5'-CAGACCCCAAGGTAGCTCAG-3' (SEQ ID NO:33);
HDNH - 5'-GGAAGACCCAGAGCAAGATC-3' (SEQ ID NO:34).

Amplified product were subject to direct sequencing after purification from an agarose gel or cloned into a TOPO PCR cloning vector (Invitrogen) for sequencing.

Multiple sequence alignment of NHL to known helicases showed that NHL contains all the seven critical helicase domains. BLAST analysis of the predicted 1,219 amino acid sequence (see Figure 2, SEQ ID NO:2) reveal an approximately 26% sequence identity and 48% sequence similarity to the RAD3/ERCC2 gene family of DNA helicases (see Figure 3). Review of this sequence data shows that two partial human cDNA clones (Acc. No. a1080127 and ab029011) are deposited. No. a1080127 covers exon 25-35 while ab029011 covers exons 9-35. Ab029011 starts at amino acid 240 of the full length human NHL protein disclosed herein, but also differs at exon 35 and appears to be a fusion transcript with M68. This cDNA was isolated from brain tissue, which has been known to express rare transcripts.

EXAMPLE 2

Northern Analysis of human NHL Expression

Messenger RNA (mRNA) obtained from human brain, heart, skeletal muscle, colon, thymus, spleen, kidney, liver, small intestine, placenta, lung, and peripheral blood leukocytes. Two μg of polyA$^+$ RNA were run on each lane a denaturing formaldehyde 1% agarose gel, and transferred to a charged-modified nylon membrane. The probe was made using a 733 bp fragment derived from 1174-1907 nt of the NHL cDNA. This fragment was labeled via the ^{32}P dCTP random priming method (Ambion). Hybridization was carried in ExpressHyb (Clontech) according to the manufacturer's protocol except for the final wash, which was at 55°C. Membranes were exposed to X-ray film with intensifying screen at -80°C overnight. The Northern data is presented in Figure 4. Note hybridization of the NHL probe to an approximately 4.4 kb transcript. The 7.5 kb transcript may suggest an alternative splicing of the NHL RNA.

EXAMPLE 3

Chromosomal localization

To map the position of M68/NHL in the human genome, primers C68.36F and C68.275R, were used to carry out PCR reactions to 93 clones of the MIT GeneBridge 4 panel (Research Genetics) and results were submitted to MIT for analysis. M68/DcR3 was mapped to the extreme telomere of chromosome 20, at 20q13.3, 28cR from D20S173 with a lod score of 13. An analogous procedure was also carried out with the 83 clones of the Stanford G3 radiation hybrid panel, with PCR results submitted to the Stanford Genome Center for analysis. Analysis using another pair of PCR primers specific to NHL yielded the same result. For fluorescence in situ (FISH) analysis, the normal human male fibroblast cell line, L136 (Coriell Cell Repository, Camden, NJ) was arrested in mitosis with colcemid (10 μg/ml). A human chromosome 20 α-satellite probe (Vysis, Downers Grove, IL) was directly labeled with Spectrum Orange dUTP and was used to identify chromosome 20. The M68 BAC clone was directly labeled with SpectrumGreen dUTP by nick translation (Vysis). Slides were counterstained with DAPI stain and viewed under an Olympus microscope with narrow blue and DAPI/TRITC filters. Fifty metaphase cells were scored to verify that the M68 probe was located on the same chromosome as the Human Chromosome 20 probe. Radiation hybrid chromosomal mapping reconfirms that it is linked to M68 locus,at 20q13.3.

WHAT IS CLAIMED IS:

1. A purified DNA molecule encoding a mammalian NHL protein.

2. A purified DNA molecule of claim 1 encoding a human NHL protein which comprises the amino acid sequence

MPKIVLNGVT VDFPFQPYKC QQEYMTKVLE CLQQKVNGIL ESPTGTGKTL CLLCTTLAWR
EHLRDGISAR KIAERAQGEL FPDRALSSWG NAAAAAGDPI ACYTDIPKII YASRTHSQLT
QVINELRNTS YRPKVCVLGS REQLCIHPEV KKQESNHLQI HLCRKKVASR SCHFYNNVEE
KSLEQELASP ILDIEDLVKS GSKHRVCPYY LSRNLKQQAD IIFMPYNYLL DAKSRRAHNI
DLKGTVVIFD EAHNVEKMCE ESASFDLTPH DLASGLDVID QVLEEQTKAA QQGEPHPEFS
ADSPSPGLNM ELEDIAKLKM ILLRLEGAID AVELPGDDSG VTKPGSYIFE LFAEAQITFQ
TKGCILDSLD QIIQHLAGRA GVFTNTAGLQ KLADIIQIVF SVDPSEGSPG SPAGLGALQS
YKVHIHPDAG HRRTAQRSDA WSTTAARKRG KVLSYWCFSP GHSMHELVRQ GVRSLILTSG
TLAPVSSFAL EMQIPFPVCL ENPHIIDKHQ IWVGVVPRGP DGAQLSSAFD RRFSEECLSS
LGKALGNIAR VVPYGLLIFF PSYPVMEKSL EFWRARDLAR KMEALKPLFV EPRSKGSFSE
TISAYYARVA APGSTGATFL AVCRGKASEG LDFSDTNGRG VIVTGLPYPP RMDPRVVLKM
QFLDEMKGQG GAGGQFLSGQ EWYRQQASRA VNQAIGRVIR HRQDYGAVFL CDHRFAFADA
RAQLPSWVRP HVRVYDNFGH VIRDVAQFFR VAERTMPAPA PRATAPSVRG EDAVSEAKSP
GPFFSTRKAK SLDLHVPSLK QRSSGSPAAG DPESSLCVEY EQEPVPARQR PRGLLAALEH
SEQRAGSPGE EQAHSCSTLS LLSEKRPAEE PRGGRKKIRL VSHPEEPVAG AQTDRAKLFM
VAVKQELSQA NFATFTQALQ DYKGSDDFAA LAACLGPLFA EDPKKHNLLQ GFYQFVRPHH
KQQFEEVCIQ LTGRGCGYRP EHSIPRRQRA QPVLDPTGRT APDPKLTVST AAAQQLDPQE
HLNQGRPHLS PRPPPTGDPG SQPQWGSGVP RAGKQGQHAV SAYLADARRA LGSAGCSQLL
AALTAYKQDD DLDKVLAVLA ALTTAKPEDF PLLHRFSMFV RPHHKQRFSQ TCTDLTGRPY
PGMEPPGPQE ERLAVPPVLT HRAPQPGPSR SEKTGKTQSK ISSFLRQRPA GTVGAGGEDA
GPSQSSGPPH GPAASEWGL* (SEQ ID NO:2).

3. An expression vector for expressing a NHL protein in a recombinant host cell wherein said expression vector comprises a DNA molecule of claim 2.

4. A host cell which expresses a recombinant NHL protein wherein said host cell contains the expression vector of claim 3.

5. A process for expressing a NHL protein in a recombinant host cell, comprising:

(a) transfecting the expression vector of claim 3 into a suitable host cell; and,

(b) culturing the host cells of step (a) under conditions which allow expression of said NHL protein from said expression vector.

6. A purified DNA molecule encoding a human NHL protein which consists of the amino acid sequence

MPKIVLNGVT VDFPFQPYKC QQEYMTKVLE CLQQKVNGIL ESPTGTGKTL CLLCTTLAWR
EHLRDGISAR KIAERAQGEL FPDRALSSWG NAAAAAGDPI ACYTDIPKII YASRTHSQLT
QVINELRNTS YRPKVCVLGS REQLCIHPEV KKQESNHLQI HLCRKKVASR SCHFYNNVEE
KSLEQELASP ILDIEDLVKS GSKHRVCPYY LSRNLKQQAD IIFMPYNYLL DAKSRRAHNI
DLKGTVVIFD EAHNVEKMCE ESASFDLTPH DLASGLDVID QVLEEQTKAA QQGEPHPEFS
ADSPSPGLNM ELEDIAKLKM ILLRLEGAID AVELPGDDSG VTKPGSYIFE LFAEAQITFQ
TKGCILDSLD QIIQHLAGRA GVFTNTAGLQ KLADIIQIVF SVDPSEGSPG SPAGLGALQS
YKVHIHPDAG HRRTAQRSDA WSTTAARKRG KVLSYWCFSP GHSMHELVRQ GVRSLILTSG
TLAPVSSFAL EMQIPFPVCL ENPHIIDKHQ IWVGVVPRGP DGAQLSSAFD RRFSEECLSS
LGKALGNIAR VVPYGLLIFF PSYPVMEKSL EFWRARDLAR KMEALKPLFV EPRSKGSFSE
TISAYYARVA APGSTGATFL AVCRGKASEG LDFSDTNGRG VIVTGLPYPP RMDPRVVLKM
QFLDEMKGQG GAGGQFLSGQ EWYRQQASRA VNQAIGRVIR HRQDYGAVFL CDHRFAFADA
RAQLPSWVRP HVRVYDNFGH VIRDVAQFFR VAERTMPAPA PRATAPSVRG EDAVSEAKSP
GPFFSTRKAK SLDLHVPSLK QRSSGSPAAG DPESSLCVEY EQEPVPARQR PRGLLAALEH
SEQRAGSPGE EQAHSCSTLS LLSEKRPAEE PRGGRKKIRL VSHPEEPVAG AQTDRAKLFM
VAVKQELSQA NFATFTQALQ DYKGSDDFAA LAACLGPLFA EDPKKHNLLQ GFYQFVRPHH
KQQFEEVCIQ LTGRGCGYRP EHSIPRRQRA QPVLDPTGRT APDPKLTVST AAAQQLDPQE
HLNQGRPHLS PRPPPTGDPG SQPQWGSGVP RAGKQGQHAV SAYLADARRA LGSAGCSQLL
AALTAYKQDD DLDKVLAVLA ALTTAKPEDF PLLHRFSMFV RPHHKQRFSQ TCTDLTGRPY
PGMEPPGPQE ERLAVPPVLT HRAPQPGPSR SEKTGKTQSK ISSFLRQRPA GTVGAGGEDA
GPSQSSGPPH GPAASEWGL* (SEQ ID NO:2).

7. An expression vector for expressing a NHL protein in a recombinant host cell wherein said expression vector comprises a DNA molecule of claim 6.

8. A host cell which expresses a recombinant NHL protein wherein said host cell contains the expression vector of claim 7.

9. A process for expressing a NHL protein in a recombinant host cell, comprising:

(a) transfecting the expression vector of claim 7 into a suitable host cell; and,

(b) culturing the host cells of step (a) under conditions which allow expression of said NHL protein from said expression vector.

10. A purified DNA molecule which comprises the nucleotide sequence as set forth in SEQ ID NO:1.

11. An expression vector for expressing a NHL protein in a recombinant host cell wherein said expression vector comprises a DNA molecule of claim 10.

12. A host cell which expresses a recombinant NHL protein wherein said host cell contains the expression vector of claim 11.

13. A purified DNA molecule which consists of the nucleotide sequence as set forth in SEQ ID NO:1.

14. An expression vector for expressing a NHL protein in a recombinant host cell wherein said expression vector comprises a DNA molecule of claim 13.

15. A host cell which expresses a recombinant NHL protein wherein said host cell contains the expression vector of claim 14.

16. A purified DNA molecule of claim 13 which consists of the nucleotide sequence from about nucleotide 828 to about nucleotide 4587, as set forth in SEQ ID NO:1.

17. An expression vector for expressing a NHL protein in a recombinant host cell wherein said expression vector comprises a DNA molecule of claim 16.

18. A host cell which expresses a recombinant NHL protein wherein said host cell contains the expression vector of claim 17.

19. A substantially purified NHL protein which comprises the amino acid sequence as set forth in SEQ ID NO:2.

20. A substantially purified NHL protein which consists of the amino acid sequence as set forth in SEQ ID NO:2.

21. A substantially purified NHL protein which comprises the amino acid sequence as set forth in SEQ ID NO:2, wherein said protein is a product of a DNA expression vector comprising SEQ ID NO:1 and contained within a recombinant host cell.

22. A method of identifying modulators of NHL activity, comprising:

(a) combining a test compound with a NHL protein, wherein NHL comprises the amino acid sequence as set forth in SEQ ID NO:2; and,

(b) measuring the effect of the test compound on the NHL protein.

23. An isolated DNA molecule which comprises the nucleotide sequence as set forth in SEQ ID NO:3.

24. An isolated DNA molecule of claim 20 which comprises from about nucleotide 47000 to about nucleotide 85500 of SEQ ID NO:3.

25. An isolated DNA molecule of claim 23 which comprises from about nucleotide 47095 to about nucleotide 85316 of SEQ ID NO:3.

26. A substantially purified NHL protein of claim 21 wherein said protein is a product of a DNA expression vector comprising from about nucleotide 828 to nucleotide 4587, as set forth in SEQ ID NO:1, and contained within a recombinant host cell.

AGTCAGCCCT GCTGCCAGCC AGTGCCGGGT GCTGGGGACT CAGGGAGGCC CGCCGGGACC ACTGCGGGAC
AGTGAGCCGA GCAGAAGCTG GAACGCAGGA GAGGAAGGAG AGGGGGCGGT CAGGGCTCTC AGGAGCCGGG
TCCTGGGCAA GGCGCAGCCG TTTTCAAATT TTCAGGAAAG CGGTCGGCTC ACACTCGAGC AGTAAAAAGA
TGCCTCTGGG GAGGAGGCCC GTGCAGCTCT CCGGGCAATG GTGGTGGCTC GGCCTAGAGA GGCGGTAGTG
GAACGCAGAC CCTGGTGGGG GAATGACATC AAGGGAGGAG ACGGGCGGGA CCCCAGATTT CTGCCTGTGG
GCGATGGAAG TGAGGTTCAC TGGCCAGCGG AGCCGGACAC AGAACGCGCA AAACGCCGTG TAGGCCTGGA
GGAGCCGAAG AGCAGGCGGA CCCCCTCCGC GGGGGAACAG TTTCCGCCGG GAGCACAAAG CAACGGACCG
GAAGTGGGGG GCGGAAGTGC AGTGGGCTCA GCGCCGACTG CGCGCCTCTG CCCGCGAAAA CTCTGAGCTG
GCTGACAGCT GGGGACGGGT GGCGGCCCTC GACTGGAGTC GGTTGAGTTC CTGAGGGACC CCGGTTCTGG
AAGGTTCGCC GCGGAGACAA GTGAGCAGTC TGTGCCATAG GGATTCTCGA AGAGAACAGC GTTGTGTCCC
AGTGCACATG CTCGCATCGC TTACCAGGAG TGCCCGAGAC CCTAAGATGT TCGGAGTGGT TTTTTCGCAC
AGACCCGAAT AGCCTGCCCC TCAGCCACGC TCTGTGCCCT TCTGAGAACA GGCTGATATG CCCAAGATAG
TCCTGAATGG TGTGACCGTA GACTTCCCTT TCCAGCCCTA CAAATGCCAA CAGGAGTACA TGACCAAGGT
CCTGGAATGT CTGCAGCAGA AGGTGAATGG CATCCTGGAG AGCCCTACGG GTACAGGGAA GACGCTGTGC
CTGCTGTGCA CCACGCTGGC CTGGCGAGAA CACCTCCGAG ACGGCATCTC TGCCCGCAAG ATTGCCGAGA
GGGCGCAAGG AGAGCTTTTC CCGGATCGGG CCTTGTCATC CTGGGGCAAC GCTGCTGCTG CTGCTGGAGA
CCCCATAGCT TGCTACACGG ACATCCCAAA GATTATTTAC GCCTCCAGGA CCCACTCGCA ACTCACACAG
GTCATCAACG AGCTTCGGAA CACCTCCTAC CGGCCTAAGG TGTGTGTGCT GGGCTCCCGG GAGCAGCTGT
GCATCCATCC TGAGGTGAAG AAACAAGAGA GTAACCATCT ACAGATCCAC TTGTGCCGTA AGAAGGTGGC
AAGTCGCTCC TGTCATTTCT ACAACAACGT AGAAGAAAAA AGCCTGGAGC AGGAGCTGGC CAGCCCCATC
CTGGACATTG AGGACTTGGT CAAGAGCGGA AGCAAGCACA GGGTGTGCCC TTACTACCTG TCCCGGAACC
TGAAGCAGCA AGCCGACATC ATATTCATGC CGTACAATTA CTTGTTGGAT GCCAAGAGCC GCAGAGCACA
CAACATTGAC CTGAAGGGGA CAGTCGTGAT CTTTGACGAA GCTCACAACG TGGAGAAGAT GTGTGAAGAA
TCGGCATCCT TTGACCTGAC TCCCCATGAC CTGGCTTCAG GACTGGACGT CATAGACCAG GTGCTGGAGG
AGCAGACCAA GGCAGCGCAG CAGGGTGAGC CCCACCCGGA GTTCAGCGCG GACTCCCCCA GCCCAGGGCT
GAACATGGAG CTGGAAGACA TTGCAAAGCT GAAGATGATC CTGCTGCGCC TGGAGGGGGC CATCGATGCT
GTTGAGCTGC CTGGAGACGA CAGCGGTGTC ACCAAGCCAG GGAGCTACAT CTTTGAGCTG TTTGCTGAAG
CCCAGATCAC GTTTCAGACC AAGGGCTGCA TCCTGGACTC GCTGGACCAG ATCATCCAGC ACCTGGCAGG
ACGTGCTGGA GTGTTCACCA ACACGGCCGG ACTGCAGAAG CTGGCGGACA TTATCCAGAT TGTGTTCAGT
GTGGACCCCT CCGAGGGCAG CCCTGGTTCC CCAGCAGGGC TGGGGGCCTT ACAGTCCTAT AAGGTGCACA
TCCATCCTGA TGCTGGTCAC CGGAGGACGG CTCAGCGGTC TGATGCCTGG AGCACCACTG CAGCCAGAAA
GCGAGGGAAG GTGCTGAGCT ACTGGTGCTT CAGTCCCGGC CACAGCATGC ACGAGCTGGT CCGCCAGGGC
GTCCGCTCCC TCATCCTTAC CAGCGGCACG CTGGCCCCGG TGTCCTCCTT TGCTCTGGAG ATGCAGATCC
CTTTCCCAGT CTGCCTGGAG AACCCACACA TCATCGACAA GCACCAGATC TGGGTGGGGG TCGTCCCCAG
AGGCCCCGAT GGAGCCCAGT TGAGCTCCGC GTTTGACAGA CGGTTTTCCG AGGAGTGCTT ATCCTCCCTG
GGGAAGGCTC TGGGCAACAT CGCCCGCGTG GTGCCCTATG GGCTCCTGAT CTTCTTCCCT TCCTATCCTG
TCATGGAGAA GAGCCTGGAG TTCTGGCGGG CCCGCGACTT GGCCAGGAAG ATGGAGGCGC TGAAGCCGCT
GTTTGTGGAG CCCAGGAGCA AAGGCAGCTT CTCCGAGACC ATCAGTGCTT ACTATGCAAG GGTTGCCGCC
CCTGGGTCCA CCGGCGCCAC CTTCCTGGCG GTCTGCCGGG GCAAGGCCAG CGAGGGGCTG GACTTCTCAG
ACACGAATGG CCGTGGTGTG ATTGTCACGG GCCTCCCGTA CCCCCCACGC ATGGACCCCC GGGTTGTCCT
CAAGATGCAG TTCCTGGATG AGATGAAGGG CCAGGGTGGG GCTGGGGGCC AGTTCCTCTC TGGGCAGGAG
TGGTACCGGC AGCAGGCGTC CAGGGCTGTG AACCAGGCCA TCGGGCGAGT GATCCGGCAC CGCCAGGACT
ACGGAGCTGT CTTCCTCTGT GACCACAGGT TCGCCTTTGC CGACGCAAGA GCCCAACTGC CCTCCTGGGT
GCGTCCCCAC GTCAGGGTGT ATGACAACTT TGGCCATGTC ATCCGAGACG TGGCCCAGTT CTTCCGTGTT
GCCGAGCGAA CTATGCCAGC GCCGGCCCCC CGGGCTACAG CACCCAGTGT GCGTGGAGAA GATGCTGTCA
GCGAGGCCAA GTCGCCTGGC CCCTTCTTCT CCACCAGGAA AGCTAAGAGT CTGGACCTGC ATGTCCCCAG
CCTGAAGCAG AGGTCCTCAG GGTCACCAGC TGCCGGGGAC CCCGAGAGTA GCCTGTGTGT GGAGTATGAG
CAGGAGCCAG TTCCTGCCCG GCAGAGGCCC AGGGGGCTGC TGGCCGCCCT GGAGCACAGC GAACAGCGGG

FIG.1A

CGGGGAGCCC TGGCGAGGAG CAGGCCCACA GCTGCTCCAC CCTGTCCCTC CTGTCTGAGA
AGAGGCCGGC AGAAGAACCG CGAGGAGGGA GGAAGAAGAT CCGGCTGGTC AGCCACCCGG
AGGAGCCCGT GGCTGGTGCA CAGACGGACA GGGCCAAGCT CTTCATGGTG GCCGTGAAGC
AGGAGTTGAG CCAAGCCAAC TTTGCCACCT TCACCCAGGC CCTGCAGGAC TACAAGGGTT
CCGATGACTT CGCCGCCCTG GCCGCCTGTC TCGGCCCCCT CTTTGCTGAG GACCCCAAGA
AGCACAACCT GCTCCAAGGC TTCTACCAGT TTGTGCGGCC CCACCATAAG CAGCAGTTTG
AGGAGGTCTG TATCCAGCTG ACAGGACGAG GCTGTGGCTA TCGGCCTGAG CACAGCATTC
CCCGAAGGCA GCGGGCACAG CCGGTCCTGG ACCCCACTGG AAGAACGGCG CCGGATCCCA
AGCTGACCGT GTCCACGGCT GCAGCCCAGC AGCTGGACCC CCAAGAGCAC CTGAACCAGG
GCAGGCCCCA CCTGTCGCCC AGGCCACCCC CAACAGGAGA CCCTGGCAGC CAACCACAGT
GGGGGTCTGG AGTGCCCAGA GCAGGGAAGC AGGGCCAGCA CGCCGTGAGC GCCTACCTGG
CTGATGCCCG CAGGGCCCTG GGGTCCGCGG GCTGTAGCCA ACTCTTGGCA GCGCTGACAG
CCTATAAGCA AGACGACGAC CTCGACAAGG TGCTGGCTGT GTTGGCCGCC CTGACCACTG
CAAAGCCAGA GGACTTCCCC CTGCTGCACA GGTTCAGCAT GTTTGTGCGT CCACACCACA
AGCAGCGCTT CTCACAGACG TGCACAGACC TGACCGGCCG GCCCTACCCG GGCATGGAGC
CACCGGGACC CCAGGAGGAG AGGCTTGCCG TGCCTCCTGT GCTTACCCAC AGGGCTCCCC
AACCAGGCCC CTCACGGTCC GAGAAGACCG GGAAGACCCA GAGCAAGATC TCGTCCTTCC
TTAGACAGAG GCCAGCAGGG ACTGTGGGGG CGGGCGGTGA GGATGCAGGT CCCAGCCAGT
CCTCAGGACC TCCCCACGGG CCTGCAGCAT CTGAGTGGGG CCTCTAGGAT GTGCCCAGCC
TGCCACACCG CCTCCAGGAA GCAGAGCGTC ATGCAGGTCT TCTGGCCAGA GCCCCAGTGA
GTGCCCACGG AGGCCCCCAG CACACCCAAC GTGGCTTGAT CACCTGCCTG TCCAGCTCTG
GTGGGCCAAG AACCCACCCA ACAGAATAGG CCAGCCCATG CCAGCCGGCT TGGCCCGCTG
CAGGCCTCAG GCAGGCGGGG CCCATGGTTG GTCCCTGCGG TGGGACCGGA TCTGGGCCTG
CCTCTGAGAA GCCCTGAGCT ACCTTGGGGT CTGGGGTGGG TTTCTGGGAA AGTGCTTCCC
CAGAACTTCC CTGGCTCCTG GCCTGTGAGT GGTGCCACAG GGGCACCCCA GCTGAGCCCC
TCACCGGGAA GGAGGAGACC CCCGTGGGCA CGTGTCCACT TTTAATCAGG GGACAGGGCT
CTCTAATAAA GCTGCTGGCA GTGCCC (SEQ ID NO:1).

FIG.1B

MPKIVLNGVT VDFPFQPYKC QQEYMTKVLE CLQQKVNGIL ESPTGTGKTL CLLCTTLAWR
EHLRDGISAR KIAERAQGEL FPDRALSSWG NAAAAAGDPI ACYTDIPKII YASRTHSQLT
QVINELRNTS YRPKVCVLGS REQLCIHPEV KKQESNHLQI HLCRKKVASR SCHFYNNVEE
KSLEQELASP ILDIEDLVKS GSKHRVCPYY LSRNLKQQAD IIFMPYNYLL DAKSRRAHNI
DLKGTVVIFD EAHNVEKMCE ESASFDLTPH DLASGLDVID QVLEEQTKAA QQGEPHPEFS
ADSPSPGLNM ELEDIAKLKM ILLRLEGAID AVELPGDDSG VTKPGSYIFE LFAEAQITFQ
TKGCILDSLD QIIQHLAGRA GVFTNTAGLQ KLADIIQIVF SVDPSEGSPG SPAGLGALQS
YKVHIHPDAG HRRTAQRSDA WSTTAARKRG KVLSYWCFSP GHSMHELVRQ GVRSLILTSG
TLAPVSSFAL EMQIPFPVCL ENPHIIDKHQ IWVGVVPRGP DGAQLSSAFD RRFSEECLSS
LGKALGNIAR VVPYGLLIFF PSYPVMEKSL EFWRARDLAR KMEALKPLFV EPRSKGSFSE
TISAYYARVA APGSTGATFL AVCRGKASEG LDFSDTNGRG VIVTGLPYPP RMDPRVVLKM
QFLDEMKGQG GAGGQFLSGQ EWYRQQASRA VNQAIGRVIR HRQDYGAVFL CDHRFAFADA
RAQLPSWVRP HVRVYDNFGH VIRDVAQFFR VAERTMPAPA PRATAPSVRG EDAVSEAKSP
GPFFSTRKAK SLDLHVPSLK QRSSGSPAAG DPESSLCVEY EQEPVPARQR PRGLLAALEH
SEQRAGSPGE EQAHSCSTLS LLSEKRPAEE PRGGRKKIRL VSHPEEPVAG AQTDRAKLFM
VAVKQELSQA NFATFTQALQ DYKGSDDFAA LAACLGPLFA EDPKKHNLLQ GFYQFVRPHH
KQQFEEVCIQ LTGRGCGYRP EHSIPRRQRA QPVLDPTGRT APDPKLTVST AAAQQLDPQE
HLNQGRPHLS PRPPPTGDPG SQPQWGSGVP RAGKQGQHAV SAYLADARRA LGSAGCSQLL
AALTAYKQDD DLDKVLAVLA ALTTAKPEDF PLLHRFSMFV RPHHKQRFSQ TCTDLTGRPY
PGMEPPGPQE ERLAVPPVLT HRAPQPGPSR SEKTGKTQSK ISSFLRQRPA GTVGAGGEDA
GPSQSSGPPH GPAASEWGL* (SEQ ID NO:2).

FIG.2

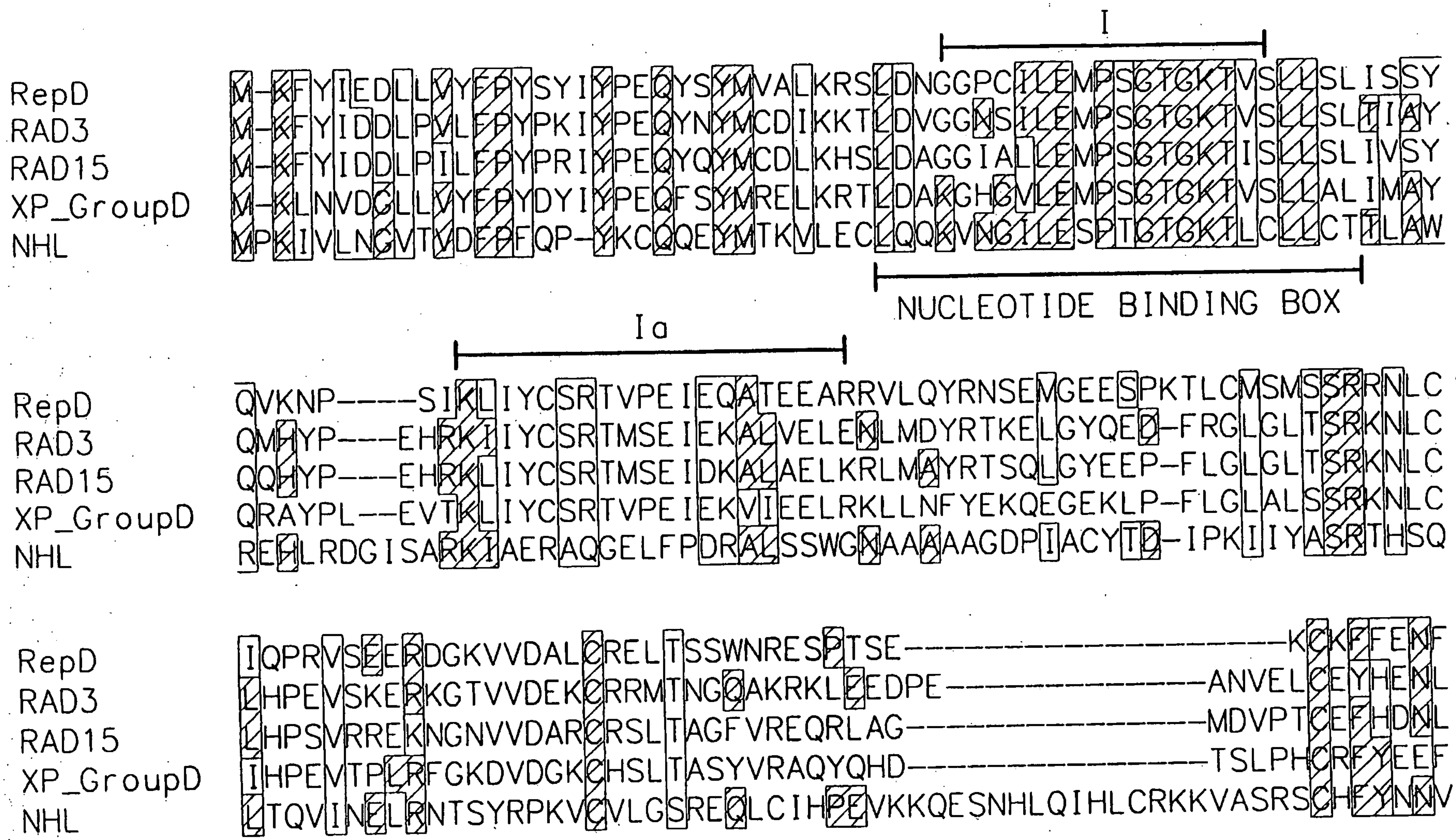
I
RepD M-KFYIEDLLVYFPYSYIYPEQYSYMVALKRSLDNGGPCILEMPSGTGKTVSLLSLISSY
RAD3 M-KFYIDDLPVLFPYPKIYPEQYNYMCDIKKTLDVGGNSILEMPSGTGKTVSLLSLTIAY
RAD15 M-KFYIDDLPILFPYPRIYPEQYQYMCDLKHSLDAGGIALLEMPSGTGKTISLLSLIVSY
XP_GroupD M-KLNVDGLLVYFPYDYIYPEQFSYMRELKRTLDAKGHGVLEMPSGTGKTVSLLALIMAY
NHL MPKIVLNGVTVDFPFQP-YKCQQEYMTKVLECLQQKVNGILESPTGTGKTLCLLCTTLAW
NUCLEOTIDE BINDING BOX
Ia
RepD QVKNP-----SIKLIYCSRTVPEIEQATEEARRVLQYRNSEMGEESPKTLCMSMSSRRNLC
RAD3 QMHYP---EHRKIIYCSRTMSEIEKALVELENLMDYRTKELGYQED-FRGLGLTSRKNLC
RAD15 QQHYP---EHRKLIYCSRTMSEIDKALAELKRLMAYRTSQLGYEEP-FLGLGLTSRKNLC
XP_GroupD QRAYPL--EVTKLIYCSRTVPEIEKVIEELRKLLNFYEKQEGEKLP-FLGLALSSRKNLC
NHL REHLRDGISARKIAERAQGELFPDRALSSWGNAAAAAGDPIACYTD-IPKIIYASRTHSQ
RepD IQPRVSEERDGKVVDALCRELTSSWNRESPTSE------------------KCKFFENF
RAD3 LHPEVSKERKGTVVDEKCRRMTNGQAKRKLEEDPE-----------ANVELCEYHENL
RAD15 LHPSVRREKNGNVVDARCRSLTAGFVREQRLAG-------------MDVPTCEFHDNL
XP_GroupD IHPEMTPLRFGKDVDGKCHSLTASYVRAQYQHD-------------TSLPHCRFYEEF
NHL LTQVINELRNTSYRPKVCVLGSREQLCIHPEVKKQESNHLQIHLCRKKVASRSCHFYNNV

FIG. 3A

```
RepD        ESNGKEILL-EGVYSLEDLKEYGLKHQMCPYFLSRHMLNFANIVIFSYQYLLDPKIASLI
RAD3        YNIEVEDYLPKGVFSFEKLLKYCEEKTLCPYFIVRRMISLCNLIIYSYHYLLDPKIAERV
RAD15       EDLEPHSLISNGVWTLDDITEYGEKTTRCPYFTVRRMLPFCNVLIYSYHYLLDPKIAERV
XP_GroupD   DAHGREVPLPAGIYNLDDLKALGRRQGWCPYFLARYSILHANVVVYSYHYLLDPKIADLV
NHL         EEKSLEQELASPILDIEDLVKSGSKHRVCPYYLSRNLKQQADIIFMPYNYLLDAKSRRAH

                  II
RepD        SSSFPSNSIVVFDEAHNIDNVCINALSINIDNKLLDTSSKNIAKINKQIEDIKKVDEKRL
RAD3        SNEVSKDSIVIFDEAHNIDNVCIESLSLDLTTDALRRATRGANALDERISEVRKVDSQKL
RAD15       SRELSKDCIVVFDEAHNIDNVCIESLSIDLTESSLRKASKSILSLEQKVNEVKQSDSKKL
XP_GroupD   SKELARKAVVVFDEAHNIDNVCIDSMSVNLTRRTLDRCQGNLETLQKTVLRIKETDEQRL
NHL         NIDLKG-TVVIFDEAHNVEKMCEESASFDLTPHDLASGLDVIDQVLEEQTKAAQQGEP--

RepD        KDEYQRLVNGLARSGSTRA--DETTSDPVLPNDVIQEAVPGNIRKPSIFISLLRRVVDYL
RAD3        QDEYEKLVQGLHSADILTDQEEPFVETPVLPQDLLTEAIPGNIRRAEHFVSFLKRLIEYL
RAD15       QDEYQKLVRGLQDANAAND-EDQFMANPVLPEDVLKEAVPGNIRRAEHFIAFLKRFVEYL
XP_GroupD   RDEYRRLVEGLREASAARE-TDAHLANPVLPDEVLQEAVPGSIRTAEHFLGFLRRLLEYV
NHL         HPEFSADSPSPGLNMELEDIAKLKMILLRLEGAIDAVELPGDDSGVTKPGSYIFELFAEA
```

FIG. 3B

```
REPD       KSRLKSQMLLSESPLAFLQGLY--HATQISSRTLRFCSSRLSSLLRTLRINDVNQFSG-I
RAD3       KTRMKVLHVISETPKSFLQHLK--QLTFIERKPLRFCSERLSLLVRTLEVTEVEDFTA-L
RAD15      KTRMKVLHVIAETPTSFLQHVK--DITFIDKKPLRFCAERLTSLVRALQISLVEDFHS-L
XP_GroupD  KWRLRVQHVVQESPPAFLSGLA--QRVCIQRKPLRFCAERLRSLLHTLEITDLADFSP-L
NHL        QITFQTKGCILDSLDQIIQHLAGRAGVFTNTAGLQKLADIIQIVFSVDPSEGSPGSPAGL

REPD       SLIADFATLVGTYN--NGFLIIIEPYYQRQNNTYDQIFQFCCLDASIGMKPIFDK-YRSV
RAD3       KDIATFATLISTYE--EGFLLIIEPYEIENAAVPNPIMRFTCLDASIAIKPVFER-FSSV
RAD15      QQVVAFATLVATYE--RGFILILEPFETENATVPNPILRFSCLDASIAIKPVFER-FRSV
XP_GroupD  TLLANFATLVSTYA--KGFTIIIEPFDDRTPTIANPILHFSCMDASLAIKPVFER-FQSV
NHL        GALQSYKVHIHPDAGHRRTAQRSDAWSTTAARKRGKVLSYWCFSPGHSMHELVRQGVRSL

            III
REPD       VITSGTLSPLDIYTKMLNFRPTVVERLTMSLNRNCICPCILTRGSDQISISTKFDVRSDT
RAD3       IITSGTISPLDMYPRMLNFKTVLQKSYAMTLAKKSFLPMIITKGSDQVAISSRFEIRNDP
RAD15      IITSGTLSPLDMYPKMLQFNTVMQESYGMSLARNCFLPMVVTRGSDQVAISSKFEARNDP
XP_GroupD  IITSGTLSPLDIYPKILDFHPVTMATFTMTLARVCLCPMIIGRGNDQVAISSKFETREDI
NHL        ILTSGTLAPVSSFALEMQIPFPVCLENPHIIDKHQIWVGVVPRGPDGAQLSSAFDRRFSE
```

FIG. 3C

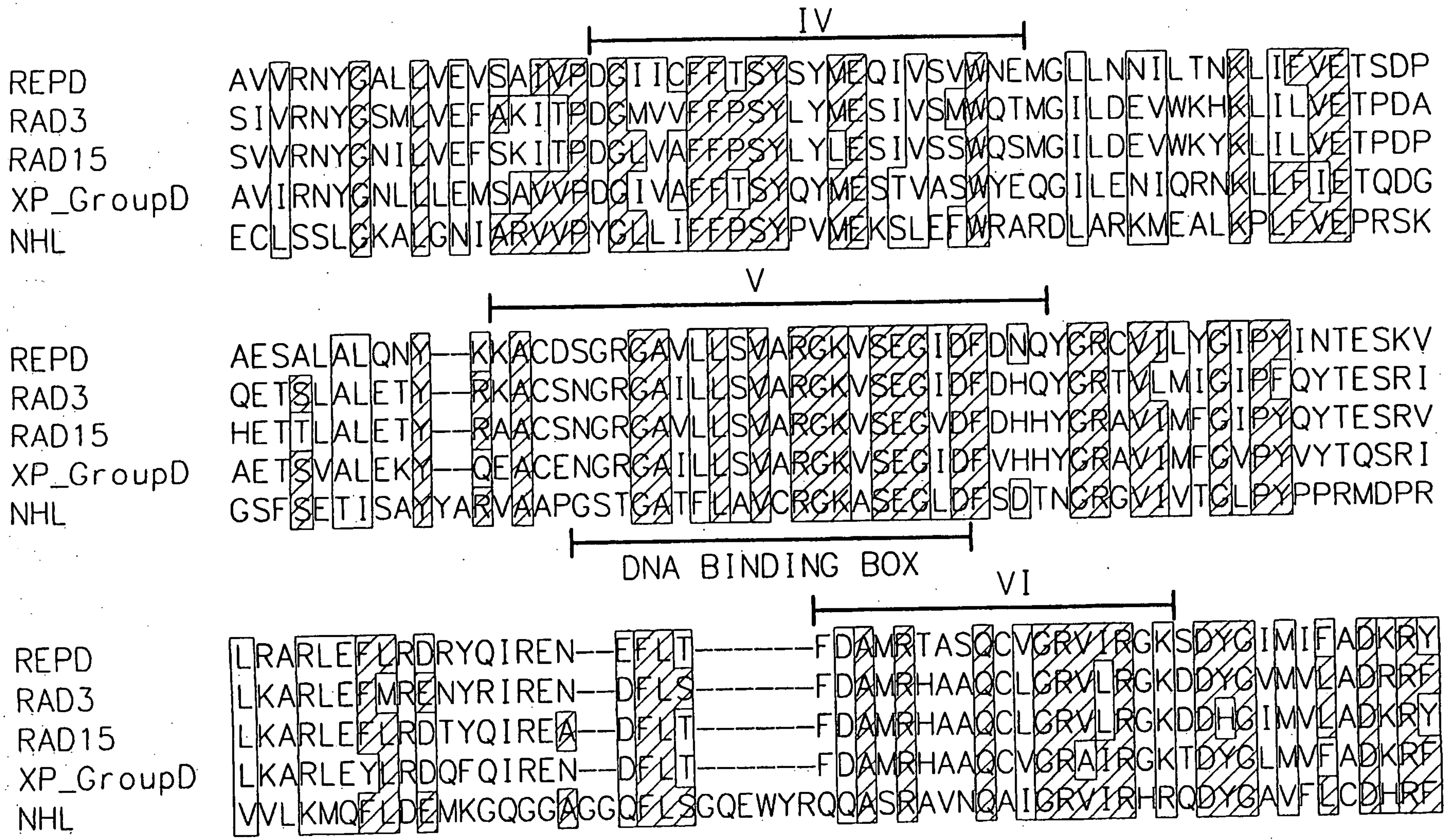
IV
REPD AVVRNYGALLVEVSAIVPDGIICFFTSYSYMEQIVSVWNEMGLLNNILTNKLIFVETSDP
RAD3 SIVRNYGSMLVEFAKITPDGMVVFFPSYLYMESIVSMWQTMGILDEVWKHKLILVETPDA
RAD15 SVVRNYGNILVEFSKITPDGLVAFFPSYLYLESIVSSWQSMGILDEVWKYKLILVETPDP
XP_GroupD AVIRNYGNLLLEMSAVVPDGIVAFFTSYQYMESTVASWYEQGILENIQRNKLLFIETQDG
NHL ECLSSLGKALGNIARVVPYGLLIFFPSYPVMEKSLEFWRARDLARKMEALKPLFVEPRSK
V
REPD AESALALQNY---KKACDSGRGAVLLSVARGKVSEGIDFDNQYGRCVILYGIPYINTESKV
RAD3 QETSLALETY---RKACSNGRGAILLSVARGKVSEGIDFDHQYGRTVLMIGIPFQYTESRI
RAD15 HETTLALETY---RAACSNGRGAVLLSVARGKVSEGVDFDHHYGRAVIMFGIPYQYTESRV
XP_GroupD AETSVALEKY---QEACENGRGAILLSVARGKVSEGIDFVHHYGRAVIMFGVPYVYTQSRI
NHL GSFSETISAYYARVAAPGSTGATFLAVCRGKASEGLDFSDTNGRGVIVTGLPYPPRMDPR
DNA BINDING BOX
VI
REPD LRARLEFLRDRYQIREN---EFLT---------FDAMRTASQCVGRVIRGKSDYGIMIFADKRY
RAD3 LKARLEFMRENYRIREN---DFLS---------FDAMRHAAQCLGRVLRGKDDYGVMVLADRRF
RAD15 LKARLEFLRDTYQIREA---DFLT---------FDAMRHAAQCLGRVLRGKDDHGIMVLADKRY
XP_GroupD LKARLEYLRDQFQIREN---DFLT---------FDAMRHAAQCVGRAIRGKTDYGLMVFADKRF
NHL VVLKMQFLDEMKGQGGAGGQFLSGQEWYRQQASRAVNQAIGRVIRHRQDYGAVFLCDHRF

FIG. 3D

```
REPD        NRLDKRNKLPQWILQFC-QPQHLNLSTDMAISLSKTFLREMGQPFSREEQLGKSLWSLEH
RAD3        SR--KRSQLPKWIAQGL-SDADLNLSTDMAISNTKQFLRTMAQPTDPKDQEGVSVWSYED
RAD15       GRSDKRTKLPKWIQQYI-TEGATNLSTDMSLALAKKFLRTMAQPFTASDQEGISWWSLDD
XP_GroupD   ARGDKRGKLPRWIQEHL-TDANLNLTVDEGVQVAKYFLRQMAQPFHREDQLGLSLLSLEQ
NHL         AFADARAQLPSWVRPHVRVYDNFGHVIRDVAQFFRVAERTMPAPAPRATAPSVRGEDAVS

REPD        VEKQSTSKPPQQQNSAINSTITTSTTTTTTTSTISETHLT (SEQ ID NO:35)
RAD3        LIKHQNS--RKDQGGFIENENKEGEQDEDEDEDIEMQ (SEQ ID NO:36)
RAD15       LLIHQK---KALKSAAIEQSKHEDEMDIDVVET (SEQ ID NO:37)
XP_GroupD   LESEETL--KRIEQIAQQL (SEQ ID NO:38)
NHL         EAKSPGPFFSTRKAKSLDLHVPSLKQRSSGSPAAGDPESSLCVEYEQEPVPARQRPRGLL
```

FIG. 3E

```
NHL    AALEHSEQRAGSPGEEQAHSCSTLSLLSEKRPAEEPRGGRKKIRLVSHPEEPVAGAQTDR
NHL    AKLFMVAVKQELSQANFATFTQALQDYKGSDDFAALAACLGPLFAEDPKKHNLLQGFYQF
NHL    VRPHHKQQFEEVCIQLTGRGCGYRPEHSIPRRQRAQPVLDPTGRTAPDPKLTVSTAAAQQ
NHL    LDPQEHLNQGRPHLSPRPPPTGDPGSQPQWGSGVPRAGKQGQHAVSAYLADARRALGSAG
NHL    CSQLLAALTAYKQDDDLDKVLAVLAALTTAKPEDFPLLHRFSMFVRPHHKQRFSQTCTDL
NHL    TGRPYPGMEPPGPQEERLAVPPVLTHRAPQPGPSRSEKTGKTQSKISSFLRQRPAGTVGA
NHL    GGEDAGPSQSSGPPHGPAASEWGL (SEQ ID NO:2)
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FIG. 3F

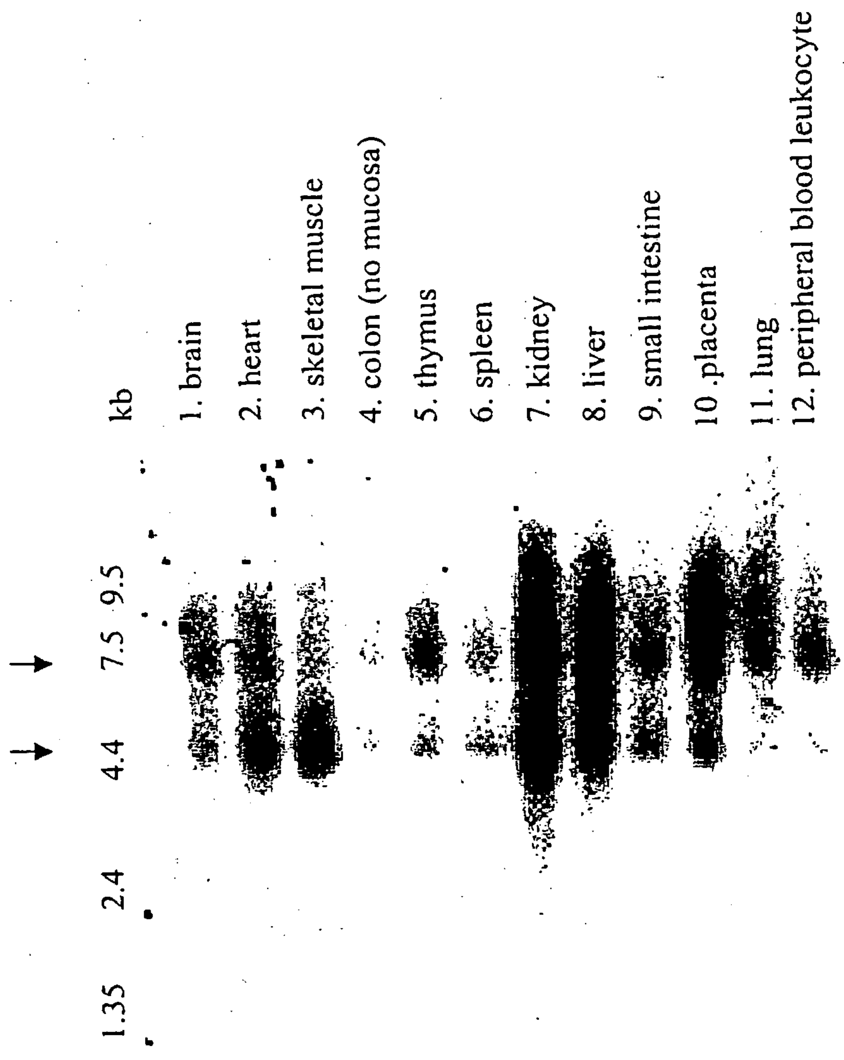

kb
1.35
2.4
4.4
7.5
9.5
1. brain
2. heart
3. skeletal muscle
4. colon (no mucosa)
5. thymus
6. spleen
7. kidney
8. liver
9. small intestine
10 .placenta
11. lung
12. peripheral blood leukocyte

FIG.4

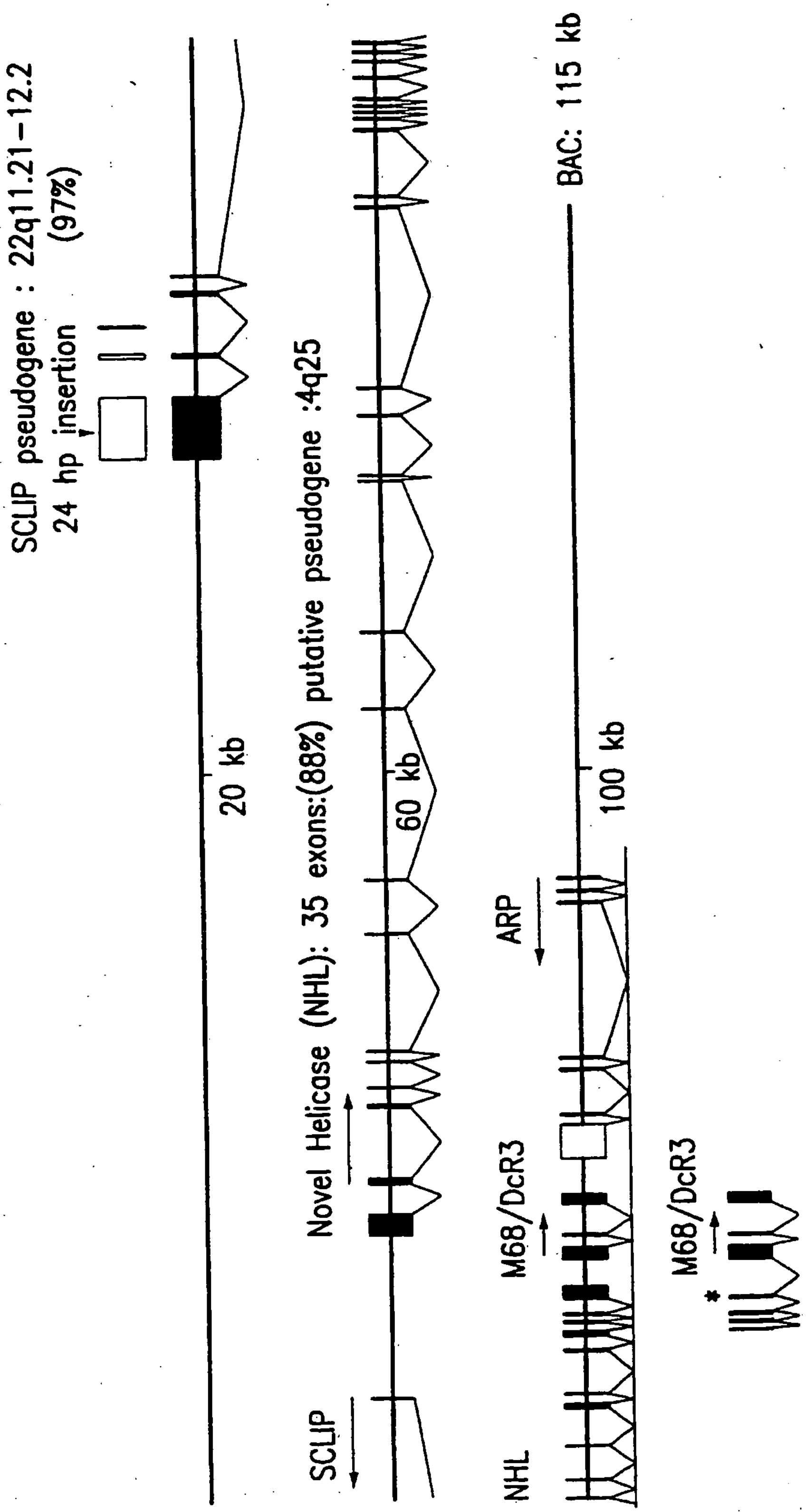
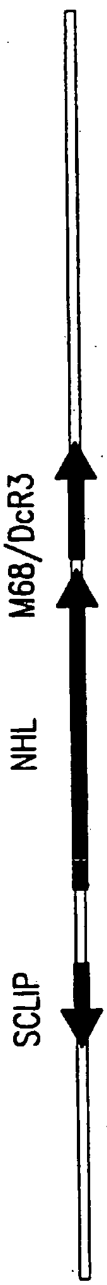

SCLIP pseudogene : 22q11.21-12.2
(97%)
24 hp insertion
20 kb
Novel Helicase (NHL): 35 exons:(88%) putative pseudogene :4q25
60 kb
BAC: 115 kb
100 kb
ARP
M68/DcR3
M68/DcR3
SCLIP
NHL

FIG.5A



Gene	Novelty	Structural family
SCLIP	known	stathmin/oncoprotein 18
NHL	novel	RAD3/ERCC2 DNA helicase
M68/DcR3	known	secreted TNFR

FIG.5B

SEQUENCE LISTING

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<120> TITLE: DNA MOLECULES ENCODING HUMAN NHL, A DNA HELICASE

<130> DOCKET/FILE REFERENCE: 20585 PCT

<160> NUMBER OF SEQUENCES: 38

<170> SOFTWARE: FastSEQ for Windows Version 4.0

<210> SEQ ID NO:1
<211> LENGTH: 4946
<212> TYPE: DNA
<213> ORGANISM:Homo sapien

<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (828)...(4487)

<400> SEQ ID NO:1

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cagggctctc aggagccggg tcctgggcaa ggcgcagccg ttttcaaatt ttcaggaaag     180
cggtcggctc acactcgagc agtaaaaaga tgcctctggg gaggaggccc gtgcagctct     240
ccgggcaatg gtggtggctc ggcctagaga ggcggtagtg gaacgcagac cctggtgggg     300
gaatgacatc aagggaggag acgggcggga ccccagattt ctgcctgtgg gcgatggaag     360
tgaggttcac tggccagcgg agccggacac agaacgcgca aaacgccgtg taggcctgga     420
ggagccgaag agcaggcgga ccccctccgc gggggaacag tttccgccgg gagcacaaag     480
caacggaccg gaagtggggg gcggaagtgc agtgggctca gcgccgactg cgcgcctctg     540
cccgcgaaaa ctctgagctg gctgacagct ggggacgggt ggcggccctc gactggagtc     600
ggttgagttc ctgagggacc ccggttctgg aaggttcgcc gcggagacaa gtgagcagtc     660
tgtgccatag ggattctcga agagaacagc gttgtgtccc agtgcacatg ctcgcatcgc     720
ttaccaggag tgcccgagac cctaagatgt tcggagtggt tttttcgcac agacccgaat     780
agcctgcccc tcagccacgc tctgtgccct tctgagaaca ggctgat atg ccc aag       836
                                                    Met Pro Lys
                                                     1

ata gtc ctg aat ggt gtg acc gta gac ttc cct ttc cag ccc tac aaa       884
Ile Val Leu Asn Gly Val Thr Val Asp Phe Pro Phe Gln Pro Tyr Lys
    5                  10                  15

tgc caa cag gag tac atg acc aag gtc ctg gaa tgt ctg cag cag aag       932
Cys Gln Gln Glu Tyr Met Thr Lys Val Leu Glu Cys Leu Gln Gln Lys
 20                  25                  30                  35

gtg aat ggc atc ctg gag agc cct acg ggt aca ggg aag acg ctg tgc       980
Val Asn Gly Ile Leu Glu Ser Pro Thr Gly Thr Gly Lys Thr Leu Cys
                40                  45                  50

ctg ctg tgc acc acg ctg gcc tgg cga gaa cac ctc cga gac ggc atc      1028
Leu Leu Cys Thr Thr Leu Ala Trp Arg Glu His Leu Arg Asp Gly Ile
            55                  60                  65

tct gcc cgc aag att gcc gag agg gcg caa gga gag ctt ttc ccg gat      1076
Ser Ala Arg Lys Ile Ala Glu Arg Ala Gln Gly Glu Leu Phe Pro Asp
        70                  75                  80
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cgg gcc ttg tca tcc tgg ggc aac gct gct gct gct gct gga gac ccc 1124
Arg Ala Leu Ser Ser Trp Gly Asn Ala Ala Ala Ala Ala Gly Asp Pro
85 90 95

ata gct tgc tac acg gac atc cca aag att att tac gcc tcc agg acc 1172
Ile Ala Cys Tyr Thr Asp Ile Pro Lys Ile Ile Tyr Ala Ser Arg Thr
100 105 110 115

cac tcg caa ctc aca cag gtc atc aac gag ctt cgg aac acc tcc tac 1220
His Ser Gln Leu Thr Gln Val Ile Asn Glu Leu Arg Asn Thr Ser Tyr
120 125 130

cgg cct aag gtg tgt gtg ctg ggc tcc cgg gag cag ctg tgc atc cat 1268
Arg Pro Lys Val Cys Val Leu Gly Ser Arg Glu Gln Leu Cys Ile His
135 140 145

cct gag gtg aag aaa caa gag agt aac cat cta cag atc cac ttg tgc 1316
Pro Glu Val Lys Lys Gln Glu Ser Asn His Leu Gln Ile His Leu Cys
150 155 160

cgt aag aag gtg gca agt cgc tcc tgt cat ttc tac aac aac gta gaa 1364
Arg Lys Lys Val Ala Ser Arg Ser Cys His Phe Tyr Asn Asn Val Glu
165 170 175

gaa aaa agc ctg gag cag gag ctg gcc agc ccc atc ctg gac att gag 1412
Glu Lys Ser Leu Glu Gln Glu Leu Ala Ser Pro Ile Leu Asp Ile Glu
180 185 190 195

gac ttg gtc aag agc gga agc aag cac agg gtg tgc cct tac tac ctg 1460
Asp Leu Val Lys Ser Gly Ser Lys His Arg Val Cys Pro Tyr Tyr Leu
200 205 210

tcc cgg aac ctg aag cag caa gcc gac atc ata ttc atg ccg tac aat 1508
Ser Arg Asn Leu Lys Gln Gln Ala Asp Ile Ile Phe Met Pro Tyr Asn
215 220 225

tac ttg ttg gat gcc aag agc cgc aga gca cac aac att gac ctg aag 1556
Tyr Leu Leu Asp Ala Lys Ser Arg Arg Ala His Asn Ile Asp Leu Lys
230 235 240

ggg aca gtc gtg atc ttt gac gaa gct cac aac gtg gag aag atg tgt 1604
Gly Thr Val Val Ile Phe Asp Glu Ala His Asn Val Glu Lys Met Cys
245 250 255

gaa gaa tcg gca tcc ttt gac ctg act ccc cat gac ctg gct tca gga 1652
Glu Glu Ser Ala Ser Phe Asp Leu Thr Pro His Asp Leu Ala Ser Gly
260 265 270 275

ctg gac gtc ata gac cag gtg ctg gag gag cag acc aag gca gcg cag 1700
Leu Asp Val Ile Asp Gln Val Leu Glu Glu Gln Thr Lys Ala Ala Gln
280 285 290

cag ggt gag ccc cac ccg gag ttc agc gcg gac tcc ccc agc cca ggg 1748
Gln Gly Glu Pro His Pro Glu Phe Ser Ala Asp Ser Pro Ser Pro Gly
295 300 305

ctg aac atg gag ctg gaa gac att gca aag ctg aag atg atc ctg ctg 1796
Leu Asn Met Glu Leu Glu Asp Ile Ala Lys Leu Lys Met Ile Leu Leu
310 315 320

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cgc ctg gag ggg gcc atc gat gct gtt gag ctg cct gga gac gac agc      1844
Arg Leu Glu Gly Ala Ile Asp Ala Val Glu Leu Pro Gly Asp Asp Ser
    325                 330                 335

ggt gtc acc aag cca ggg agc tac atc ttt gag ctg ttt gct gaa gcc      1892
Gly Val Thr Lys Pro Gly Ser Tyr Ile Phe Glu Leu Phe Ala Glu Ala
340                 345                 350                 355

cag atc acg ttt cag acc aag ggc tgc atc ctg gac tcg ctg gac cag      1940
Gln Ile Thr Phe Gln Thr Lys Gly Cys Ile Leu Asp Ser Leu Asp Gln
                360                 365                 370

atc atc cag cac ctg gca gga cgt gct gga gtg ttc acc aac acg gcc      1988
Ile Ile Gln His Leu Ala Gly Arg Ala Gly Val Phe Thr Asn Thr Ala
            375                 380                 385

gga ctg cag aag ctg gcg gac att atc cag att gtg ttc agt gtg gac      2036
Gly Leu Gln Lys Leu Ala Asp Ile Ile Gln Ile Val Phe Ser Val Asp
        390                 395                 400

ccc tcc gag ggc agc cct ggt tcc cca gca ggg ctg ggg gcc tta cag      2084
Pro Ser Glu Gly Ser Pro Gly Ser Pro Ala Gly Leu Gly Ala Leu Gln
    405                 410                 415

tcc tat aag gtg cac atc cat cct gat gct ggt cac cgg agg acg gct      2132
Ser Tyr Lys Val His Ile His Pro Asp Ala Gly His Arg Arg Thr Ala
420                 425                 430                 435

cag cgg tct gat gcc tgg agc acc act gca gcc aga aag cga ggg aag      2180
Gln Arg Ser Asp Ala Trp Ser Thr Thr Ala Ala Arg Lys Arg Gly Lys
                440                 445                 450

gtg ctg agc tac tgg tgc ttc agt ccc ggc cac agc atg cac gag ctg      2228
Val Leu Ser Tyr Trp Cys Phe Ser Pro Gly His Ser Met His Glu Leu
            455                 460                 465

gtc cgc cag ggc gtc cgc tcc ctc atc ctt acc agc ggc acg ctg gcc      2276
Val Arg Gln Gly Val Arg Ser Leu Ile Leu Thr Ser Gly Thr Leu Ala
        470                 475                 480

ccg gtg tcc tcc ttt gct ctg gag atg cag atc cct ttc cca gtc tgc      2324
Pro Val Ser Ser Phe Ala Leu Glu Met Gln Ile Pro Phe Pro Val Cys
    485                 490                 495

ctg gag aac cca cac atc atc gac aag cac cag atc tgg gtg ggg gtc      2372
Leu Glu Asn Pro His Ile Ile Asp Lys His Gln Ile Trp Val Gly Val
500                 505                 510                 515

gtc ccc aga ggc ccc gat gga gcc cag ttg agc tcc gcg ttt gac aga      2420
Val Pro Arg Gly Pro Asp Gly Ala Gln Leu Ser Ser Ala Phe Asp Arg
                520                 525                 530

cgg ttt tcc gag gag tgc tta tcc tcc ctg ggg aag gct ctg ggc aac      2468
Arg Phe Ser Glu Glu Cys Leu Ser Ser Leu Gly Lys Ala Leu Gly Asn
            535                 540                 545

atc gcc cgc gtg gtg ccc tat ggg ctc ctg atc ttc ttc cct tcc tat      2516
Ile Ala Arg Val Val Pro Tyr Gly Leu Leu Ile Phe Phe Pro Ser Tyr
        550                 555                 560
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cct gtc atg gag aag agc ctg gag ttc tgg cgg gcc cgc gac ttg gcc      2564
Pro Val Met Glu Lys Ser Leu Glu Phe Trp Arg Ala Arg Asp Leu Ala
    565                 570                 575

agg aag atg gag gcg ctg aag ccg ctg ttt gtg gag ccc agg agc aaa      2612
Arg Lys Met Glu Ala Leu Lys Pro Leu Phe Val Glu Pro Arg Ser Lys
580                 585                 590                 595

ggc agc ttc tcc gag acc atc agt gct tac tat gca agg gtt gcc gcc      2660
Gly Ser Phe Ser Glu Thr Ile Ser Ala Tyr Tyr Ala Arg Val Ala Ala
                600                 605                 610

cct ggg tcc acc ggc gcc acc ttc ctg gcg gtc tgc cgg ggc aag gcc      2708
Pro Gly Ser Thr Gly Ala Thr Phe Leu Ala Val Cys Arg Gly Lys Ala
            615                 620                 625

agc gag ggg ctg gac ttc tca gac acg aat ggc cgt ggt gtg att gtc      2756
Ser Glu Gly Leu Asp Phe Ser Asp Thr Asn Gly Arg Gly Val Ile Val
        630                 635                 640

acg ggc ctc ccg tac ccc cca cgc atg gac ccc cgg gtt gtc ctc aag      2804
Thr Gly Leu Pro Tyr Pro Pro Arg Met Asp Pro Arg Val Val Leu Lys
    645                 650                 655

atg cag ttc ctg gat gag atg aag ggc cag ggt ggg gct ggg ggc cag      2852
Met Gln Phe Leu Asp Glu Met Lys Gly Gln Gly Gly Ala Gly Gly Gln
660                 665                 670                 675

ttc ctc tct ggg cag gag tgg tac cgg cag cag gcg tcc agg gct gtg      2900
Phe Leu Ser Gly Gln Glu Trp Tyr Arg Gln Gln Ala Ser Arg Ala Val
                680                 685                 690

aac cag gcc atc ggg cga gtg atc cgg cac cgc cag gac tac gga gct      2948
Asn Gln Ala Ile Gly Arg Val Ile Arg His Arg Gln Asp Tyr Gly Ala
            695                 700                 705

gtc ttc ctc tgt gac cac agg ttc gcc ttt gcc gac gca aga gcc caa      2996
Val Phe Leu Cys Asp His Arg Phe Ala Phe Ala Asp Ala Arg Ala Gln
        710                 715                 720

ctg ccc tcc tgg gtg cgt ccc cac gtc agg gtg tat gac aac ttt ggc      3044
Leu Pro Ser Trp Val Arg Pro His Val Arg Val Tyr Asp Asn Phe Gly
    725                 730                 735

cat gtc atc cga gac gtg gcc cag ttc ttc cgt gtt gcc gag cga act      3092
His Val Ile Arg Asp Val Ala Gln Phe Phe Arg Val Ala Glu Arg Thr
740                 745                 750                 755

atg cca gcg ccg gcc ccc cgg gct aca gca ccc agt gtg cgt gga gaa      3140
Met Pro Ala Pro Ala Pro Arg Ala Thr Ala Pro Ser Val Arg Gly Glu
                760                 765                 770

gat gct gtc agc gag gcc aag tcg cct ggc ccc ttc ttc tcc acc agg      3188
Asp Ala Val Ser Glu Ala Lys Ser Pro Gly Pro Phe Phe Ser Thr Arg
            775                 780                 785

aaa gct aag agt ctg gac ctg cat gtc ccc agc ctg aag cag agg tcc      3236
Lys Ala Lys Ser Leu Asp Leu His Val Pro Ser Leu Lys Gln Arg Ser
        790                 795                 800
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tca ggg tca cca gct gcc ggg gac ccc gag agt agc ctg tgt gtg gag      3284
Ser Gly Ser Pro Ala Ala Gly Asp Pro Glu Ser Ser Leu Cys Val Glu
    805                 810                 815

tat gag cag gag cca gtt cct gcc cgg cag agg ccc agg ggg ctg ctg      3332
Tyr Glu Gln Glu Pro Val Pro Ala Arg Gln Arg Pro Arg Gly Leu Leu
820                 825                 830                 835

gcc gcc ctg gag cac agc gaa cag cgg gcg ggg agc cct ggc gag gag      3380
Ala Ala Leu Glu His Ser Glu Gln Arg Ala Gly Ser Pro Gly Glu Glu
                840                 845                 850

cag gcc cac agc tgc tcc acc ctg tcc ctc ctg tct gag aag agg ccg      3428
Gln Ala His Ser Cys Ser Thr Leu Ser Leu Leu Ser Glu Lys Arg Pro
            855                 860                 865

gca gaa gaa ccg cga gga ggg agg aag aag atc cgg ctg gtc agc cac      3476
Ala Glu Glu Pro Arg Gly Gly Arg Lys Lys Ile Arg Leu Val Ser His
        870                 875                 880

ccg gag gag ccc gtg gct ggt gca cag acg gac agg gcc aag ctc ttc      3524
Pro Glu Glu Pro Val Ala Gly Ala Gln Thr Asp Arg Ala Lys Leu Phe
    885                 890                 895

atg gtg gcc gtg aag cag gag ttg agc caa gcc aac ttt gcc acc ttc      3572
Met Val Ala Val Lys Gln Glu Leu Ser Gln Ala Asn Phe Ala Thr Phe
900                 905                 910                 915

acc cag gcc ctg cag gac tac aag ggt tcc gat gac ttc gcc gcc ctg      3620
Thr Gln Ala Leu Gln Asp Tyr Lys Gly Ser Asp Asp Phe Ala Ala Leu
                920                 925                 930

gcc gcc tgt ctc ggc ccc ctc ttt gct gag gac ccc aag aag cac aac      3668
Ala Ala Cys Leu Gly Pro Leu Phe Ala Glu Asp Pro Lys Lys His Asn
            935                 940                 945

ctg ctc caa ggc ttc tac cag ttt gtg cgg ccc cac cat aag cag cag      3716
Leu Leu Gln Gly Phe Tyr Gln Phe Val Arg Pro His His Lys Gln Gln
        950                 955                 960

ttt gag gag gtc tgt atc cag ctg aca gga cga ggc tgt ggc tat cgg      3764
Phe Glu Glu Val Cys Ile Gln Leu Thr Gly Arg Gly Cys Gly Tyr Arg
    965                 970                 975

cct gag cac agc att ccc cga agg cag cgg gca cag ccg gtc ctg gac      3812
Pro Glu His Ser Ile Pro Arg Arg Gln Arg Ala Gln Pro Val Leu Asp
980                 985                 990                 995

ccc act gga aga acg gcg ccg gat ccc aag ctg acc gtg tcc acg gct      3860
Pro Thr Gly Arg Thr Ala Pro Asp Pro Lys Leu Thr Val Ser Thr Ala
                1000                1005                1010

gca gcc cag cag ctg gac ccc caa gag cac ctg aac cag ggc agg ccc      3908
Ala Ala Gln Gln Leu Asp Pro Gln Glu His Leu Asn Gln Gly Arg Pro
            1015                1020                1025

cac ctg tcg ccc agg cca ccc cca aca gga gac cct ggc agc caa cca      3956
His Leu Ser Pro Arg Pro Pro Pro Thr Gly Asp Pro Gly Ser Gln Pro
        1030                1035                1040
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cag tgg ggg tct gga gtg ccc aga gca ggg aag cag ggc cag cac gcc      4004
Gln Trp Gly Ser Gly Val Pro Arg Ala Gly Lys Gln Gly Gln His Ala
    1045                1050                1055

gtg agc gcc tac ctg gct gat gcc cgc agg gcc ctg ggg tcc gcg ggc      4052
Val Ser Ala Tyr Leu Ala Asp Ala Arg Arg Ala Leu Gly Ser Ala Gly
1060                1065                1070                1075

tgt agc caa ctc ttg gca gcg ctg aca gcc tat aag caa gac gac gac      4100
Cys Ser Gln Leu Leu Ala Ala Leu Thr Ala Tyr Lys Gln Asp Asp Asp
                1080                1085                1090

ctc gac aag gtg ctg gct gtg ttg gcc gcc ctg acc act gca aag cca      4148
Leu Asp Lys Val Leu Ala Val Leu Ala Ala Leu Thr Thr Ala Lys Pro
            1095                1100                1105

gag gac ttc ccc ctg ctg cac agg ttc agc atg ttt gtg cgt cca cac      4196
Glu Asp Phe Pro Leu Leu His Arg Phe Ser Met Phe Val Arg Pro His
        1110                1115                1120

cac aag cag cgc ttc tca cag acg tgc aca gac ctg acc ggc cgg ccc      4244
His Lys Gln Arg Phe Ser Gln Thr Cys Thr Asp Leu Thr Gly Arg Pro
    1125                1130                1135

tac ccg ggc atg gag cca ccg gga ccc cag gag gag agg ctt gcc gtg      4292
Tyr Pro Gly Met Glu Pro Pro Gly Pro Gln Glu Glu Arg Leu Ala Val
1140                1145                1150                1155

cct cct gtg ctt acc cac agg gct ccc caa cca ggc ccc tca cgg tcc      4340
Pro Pro Val Leu Thr His Arg Ala Pro Gln Pro Gly Pro Ser Arg Ser
                1160                1165                1170

gag aag acc ggg aag acc cag agc aag atc tcg tcc ttc ctt aga cag      4388
Glu Lys Thr Gly Lys Thr Gln Ser Lys Ile Ser Ser Phe Leu Arg Gln
            1175                1180                1185

agg cca gca ggg act gtg ggg gcg ggc ggt gag gat gca ggt ccc agc      4436
Arg Pro Ala Gly Thr Val Gly Ala Gly Gly Glu Asp Ala Gly Pro Ser
        1190                1195                1200

cag tcc tca gga cct ccc cac ggg cct gca gca tct gag tgg ggc ctc      4484
Gln Ser Ser Gly Pro Pro His Gly Pro Ala Ala Ser Glu Trp Gly Leu
    1205                1210                1215

tag gatgtgccca gcctgccaca ccgcctccag gaagcagagc gtcatgcagg           4537
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tcttctggcc agagccccag tgagtgccca cggaggcccc cagcacaccc aacgtggctt    4597
gatcacctgc ctgtccagct ctggtgggcc aagaacccac ccaacagaat aggccagccc    4657
atgccagccg gcttggcccg ctgcaggcct caggcaggcg gggcccatgg ttggtccctg    4717
cggtgggacc ggatctgggc ctgcctctga gaagccctga gctaccttgg ggtctggggt    4777
gggtttctgg gaaagtgctt ccccagaact tccctggctc ctggcctgtg agtggtgcca    4837
caggggcacc ccagctgagc ccctcaccgg gaaggaggag acccccgtgg gcacgtgtcc    4897
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Pro Tyr Lys Cys Gln Gln Glu Tyr Met Thr Lys Val Leu Glu Cys Leu
            20                  25                  30
Gln Gln Lys Val Asn Gly Ile Leu Glu Ser Pro Thr Gly Thr Gly Lys
        35                  40                  45
Thr Leu Cys Leu Leu Cys Thr Thr Leu Ala Trp Arg Glu His Leu Arg
    50                  55                  60
Asp Gly Ile Ser Ala Arg Lys Ile Ala Glu Arg Ala Gln Gly Glu Leu
65                  70                  75                  80
Phe Pro Asp Arg Ala Leu Ser Ser Trp Gly Asn Ala Ala Ala Ala Ala
                85                  90                  95
Gly Asp Pro Ile Ala Cys Tyr Thr Asp Ile Pro Lys Ile Ile Tyr Ala
            100                 105                 110
Ser Arg Thr His Ser Gln Leu Thr Gln Val Ile Asn Glu Leu Arg Asn
        115                 120                 125
Thr Ser Tyr Arg Pro Lys Val Cys Val Leu Gly Ser Arg Glu Gln Leu
    130                 135                 140
Cys Ile His Pro Glu Val Lys Lys Gln Glu Ser Asn His Leu Gln Ile
145                 150                 155                 160
His Leu Cys Arg Lys Lys Val Ala Ser Arg Ser Cys His Phe Tyr Asn
                165                 170                 175
Asn Val Glu Glu Lys Ser Leu Glu Gln Glu Leu Ala Ser Pro Ile Leu
            180                 185                 190
Asp Ile Glu Asp Leu Val Lys Ser Gly Ser Lys His Arg Val Cys Pro
        195                 200                 205
Tyr Tyr Leu Ser Arg Asn Leu Lys Gln Gln Ala Asp Ile Ile Phe Met
    210                 215                 220
Pro Tyr Asn Tyr Leu Leu Asp Ala Lys Ser Arg Arg Ala His Asn Ile
225                 230                 235                 240
Asp Leu Lys Gly Thr Val Val Ile Phe Asp Glu Ala His Asn Val Glu
                245                 250                 255
Lys Met Cys Glu Glu Ser Ala Ser Phe Asp Leu Thr Pro His Asp Leu
            260                 265                 270
Ala Ser Gly Leu Asp Val Ile Asp Gln Val Leu Glu Glu Gln Thr Lys
        275                 280                 285
Ala Ala Gln Gln Gly Glu Pro His Pro Glu Phe Ser Ala Asp Ser Pro
    290                 295                 300
Ser Pro Gly Leu Asn Met Glu Leu Glu Asp Ile Ala Lys Leu Lys Met
305                 310                 315                 320
Ile Leu Leu Arg Leu Glu Gly Ala Ile Asp Ala Val Glu Leu Pro Gly
                325                 330                 335
Asp Asp Ser Gly Val Thr Lys Pro Gly Ser Tyr Ile Phe Glu Leu Phe
            340                 345                 350
Ala Glu Ala Gln Ile Thr Phe Gln Thr Lys Gly Cys Ile Leu Asp Ser
        355                 360                 365
Leu Asp Gln Ile Ile Gln His Leu Ala Gly Arg Ala Gly Val Phe Thr
    370                 375                 380
Asn Thr Ala Gly Leu Gln Lys Leu Ala Asp Ile Ile Gln Ile Val Phe
385                 390                 395                 400
Ser Val Asp Pro Ser Glu Gly Ser Pro Gly Ser Pro Ala Gly Leu Gly
                405                 410                 415
Ala Leu Gln Ser Tyr Lys Val His Ile His Pro Asp Ala Gly His Arg
            420                 425                 430
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Arg Thr Ala Gln Arg Ser Asp Ala Trp Ser Thr Thr Ala Ala Arg Lys
        435                 440                 445
Arg Gly Lys Val Leu Ser Tyr Trp Cys Phe Ser Pro Gly His Ser Met
    450                 455                 460
His Glu Leu Val Arg Gln Gly Val Arg Ser Leu Ile Leu Thr Ser Gly
465                 470                 475                 480
Thr Leu Ala Pro Val Ser Ser Phe Ala Leu Glu Met Gln Ile Pro Phe
                485                 490                 495
Pro Val Cys Leu Glu Asn Pro His Ile Ile Asp Lys His Gln Ile Trp
            500                 505                 510
Val Gly Val Val Pro Arg Gly Pro Asp Gly Ala Gln Leu Ser Ser Ala
        515                 520                 525
Phe Asp Arg Arg Phe Ser Glu Glu Cys Leu Ser Ser Leu Gly Lys Ala
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Leu Gly Asn Ile Ala Arg Val Val Pro Tyr Gly Leu Leu Ile Phe Phe
545                 550                 555                 560
Pro Ser Tyr Pro Val Met Glu Lys Ser Leu Glu Phe Trp Arg Ala Arg
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Asp Leu Ala Arg Lys Met Glu Ala Leu Lys Pro Leu Phe Val Glu Pro
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Arg Ser Lys Gly Ser Phe Ser Glu Thr Ile Ser Ala Tyr Tyr Ala Arg
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Val Ala Ala Pro Gly Ser Thr Gly Ala Thr Phe Leu Ala Val Cys Arg
    610                 615                 620
Gly Lys Ala Ser Glu Gly Leu Asp Phe Ser Asp Thr Asn Gly Arg Gly
625                 630                 635                 640
Val Ile Val Thr Gly Leu Pro Tyr Pro Pro Arg Met Asp Pro Arg Val
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Val Leu Lys Met Gln Phe Leu Asp Glu Met Lys Gly Gln Gly Gly Ala
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Gly Gly Gln Phe Leu Ser Gly Gln Glu Trp Tyr Arg Gln Gln Ala Ser
        675                 680                 685
Arg Ala Val Asn Gln Ala Ile Gly Arg Val Ile Arg His Arg Gln Asp
    690                 695                 700
Tyr Gly Ala Val Phe Leu Cys Asp His Arg Phe Ala Phe Ala Asp Ala
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Arg Ala Gln Leu Pro Ser Trp Val Arg Pro His Val Arg Val Tyr Asp
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Asn Phe Gly His Val Ile Arg Asp Val Ala Gln Phe Phe Arg Val Ala
            740                 745                 750
Glu Arg Thr Met Pro Ala Pro Ala Pro Arg Ala Thr Ala Pro Ser Val
        755                 760                 765
Arg Gly Glu Asp Ala Val Ser Glu Ala Lys Ser Pro Gly Pro Phe Phe
    770                 775                 780
Ser Thr Arg Lys Ala Lys Ser Leu Asp Leu His Val Pro Ser Leu Lys
785                 790                 795                 800
Gln Arg Ser Ser Gly Ser Pro Ala Ala Gly Asp Pro Glu Ser Ser Leu
                805                 810                 815
Cys Val Glu Tyr Glu Gln Glu Pro Val Pro Ala Arg Gln Arg Pro Arg
            820                 825                 830
Gly Leu Leu Ala Ala Leu Glu His Ser Glu Gln Arg Ala Gly Ser Pro
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Gly Glu Glu Gln Ala His Ser Cys Ser Thr Leu Ser Leu Leu Ser Glu
    850                 855                 860
Lys Arg Pro Ala Glu Glu Pro Arg Gly Gly Arg Lys Lys Ile Arg Leu
865                 870                 875                 880
Val Ser His Pro Glu Glu Pro Val Ala Gly Ala Gln Thr Asp Arg Ala
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Lys Leu Phe Met Val Ala Val Lys Gln Glu Leu Ser Gln Ala Asn Phe
            900                 905                 910
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Ala Thr Phe Thr Gln Ala Leu Gln Asp Tyr Lys Gly Ser Asp Asp Phe
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Ala Ala Leu Ala Ala Cys Leu Gly Pro Leu Phe Ala Glu Asp Pro Lys
930 935 940
Lys His Asn Leu Leu Gln Gly Phe Tyr Gln Phe Val Arg Pro His His
945 950 955 960
Lys Gln Gln Phe Glu Glu Val Cys Ile Gln Leu Thr Gly Arg Gly Cys
965 970 975
Gly Tyr Arg Pro Glu His Ser Ile Pro Arg Arg Gln Arg Ala Gln Pro
980 985 990
Val Leu Asp Pro Thr Gly Arg Thr Ala Pro Asp Pro Lys Leu Thr Val
995 1000 1005
Ser Thr Ala Ala Ala Gln Gln Leu Asp Pro Gln Glu His Leu Asn Gln
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Ser Ala Gly Cys Ser Gln Leu Leu Ala Ala Leu Thr Ala Tyr Lys Gln
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Asp Asp Asp Leu Asp Lys Val Leu Ala Val Leu Ala Ala Leu Thr Thr
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Ala Lys Pro Glu Asp Phe Pro Leu Leu His Arg Phe Ser Met Phe Val
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Arg Pro His His Lys Gln Arg Phe Ser Gln Thr Cys Thr Asp Leu Thr
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1170 1175 1180
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<211> LENGTH: 114793
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Asn Lys Leu Ile Phe Val Glu Thr Ser Asp Pro Ala Glu Ser Ala Leu
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Ala Leu Gln Asn Tyr Lys Lys Ala Cys Asp Ser Gly Arg Gly Ala Val
            580                 585                 590
Leu Leu Ser Val Ala Arg Gly Lys Val Ser Glu Gly Ile Asp Phe Asp
        595                 600                 605
Asn Gln Tyr Gly Arg Cys Val Ile Leu Tyr Gly Ile Pro Tyr Ile Asn
    610                 615                 620
Thr Glu Ser Lys Val Leu Arg Ala Arg Leu Glu Phe Leu Arg Asp Arg
625                 630                 635                 640
Tyr Gln Ile Arg Glu Asn Glu Phe Leu Thr Phe Asp Ala Met Arg Thr
                645                 650                 655
Ala Ser Gln Cys Val Gly Arg Val Ile Arg Gly Lys Ser Asp Tyr Gly
            660                 665                 670
Ile Met Ile Phe Ala Asp Lys Arg Tyr Asn Arg Leu Asp Lys Arg Asn
        675                 680                 685
Lys Leu Pro Gln Trp Ile Leu Gln Phe Cys Gln Pro Gln His Leu Asn
    690                 695                 700
Leu Ser Thr Asp Met Ala Ile Ser Leu Ser Lys Thr Phe Leu Arg Glu
705                 710                 715                 720
Met Gly Gln Pro Phe Ser Arg Glu Glu Gln Leu Gly Lys Ser Leu Trp
                725                 730                 735
Ser Leu Glu His Val Glu Lys Gln Ser Thr Ser Lys Pro Pro Gln Gln
            740                 745                 750
Gln Asn Ser Ala Ile Asn Ser Thr Ile Thr Thr Ser Thr Thr Thr Thr
        755                 760                 765
Thr Thr Thr Ser Thr Ile Ser Glu Thr His Leu Thr
    770                 775                 780
```

<210> SEQ ID NO:36
<211> LENGTH: 778
<212> TYPE: PRT
<213> ORGANISM:S. cerevisiae

<400> SEQ ID NO:36

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Met Lys Phe Tyr Ile Asp Asp Leu Pro Val Leu Phe Pro Tyr Pro Lys
 1               5                  10                  15
Ile Tyr Pro Glu Gln Tyr Asn Tyr Met Cys Asp Ile Lys Lys Thr Leu
            20                  25                  30
Asp Val Gly Gly Asn Ser Ile Leu Glu Met Pro Ser Gly Thr Gly Lys
        35                  40                  45
Thr Val Ser Leu Leu Ser Leu Thr Ile Ala Tyr Gln Met His Tyr Pro
    50                  55                  60
Glu His Arg Lys Ile Ile Tyr Cys Ser Arg Thr Met Ser Glu Ile Glu
65                  70                  75                  80
Lys Ala Leu Val Glu Leu Glu Asn Leu Met Asp Tyr Arg Thr Lys Glu
                85                  90                  95
Leu Gly Tyr Gln Glu Asp Phe Arg Gly Leu Gly Leu Thr Ser Arg Lys
            100                 105                 110
Asn Leu Cys Leu His Pro Glu Val Ser Lys Glu Arg Lys Gly Thr Val
        115                 120                 125
Val Asp Glu Lys Cys Arg Arg Met Thr Asn Gly Gln Ala Lys Arg Lys
    130                 135                 140
Leu Glu Glu Asp Pro Glu Ala Asn Val Glu Leu Cys Glu Tyr His Glu
```

145 150 155 160

Asn Leu Tyr Asn Ile Glu Val Glu Asp Tyr Leu Pro Lys Gly Val Phe
165 170 175
Ser Phe Glu Lys Leu Leu Lys Tyr Cys Glu Glu Lys Thr Leu Cys Pro
180 185 190
Tyr Phe Ile Val Arg Arg Met Ile Ser Leu Cys Asn Ile Ile Ile Tyr
195 200 205
Ser Tyr His Tyr Leu Leu Asp Pro Lys Ile Ala Glu Arg Val Ser Asn
210 215 220
Glu Val Ser Lys Asp Ser Ile Val Ile Phe Asp Glu Ala His Asn Ile
225 230 235 240
Asp Asn Val Cys Ile Glu Ser Leu Ser Leu Asp Leu Thr Thr Asp Ala
245 250 255
Leu Arg Arg Ala Thr Arg Gly Ala Asn Ala Leu Asp Glu Arg Ile Ser
260 265 270
Glu Val Arg Lys Val Asp Ser Gln Lys Leu Gln Asp Glu Tyr Glu Lys
275 280 285
Leu Val Gln Gly Leu His Ser Ala Asp Ile Leu Thr Asp Gln Glu Glu
290 295 300
Pro Phe Val Glu Thr Pro Val Leu Pro Gln Asp Leu Leu Thr Glu Ala
305 310 315 320
Ile Pro Gly Asn Ile Arg Arg Ala Glu His Phe Val Ser Phe Leu Lys
325 330 335
Arg Leu Ile Glu Tyr Leu Lys Thr Arg Met Lys Val Leu His Val Ile
340 345 350
Ser Glu Thr Pro Lys Ser Phe Leu Gln His Leu Lys Gln Leu Thr Phe
355 360 365
Ile Glu Arg Lys Pro Leu Arg Phe Cys Ser Glu Arg Leu Ser Leu Leu
370 375 380
Val Arg Thr Leu Glu Val Thr Glu Val Glu Asp Phe Thr Ala Leu Lys
385 390 395 400
Asp Ile Ala Thr Phe Ala Thr Leu Ile Ser Thr Tyr Glu Glu Gly Phe
405 410 415
Leu Leu Ile Ile Glu Pro Tyr Glu Ile Glu Asn Ala Ala Val Pro Asn
420 425 430
Pro Ile Met Arg Phe Thr Cys Leu Asp Ala Ser Ile Ala Ile Lys Pro
435 440 445
Val Phe Glu Arg Phe Ser Ser Val Ile Ile Thr Ser Gly Thr Ile Ser
450 455 460
Pro Leu Asp Met Tyr Pro Arg Met Leu Asn Phe Lys Thr Val Leu Gln
465 470 475 480
Lys Ser Tyr Ala Met Thr Leu Ala Lys Lys Ser Phe Leu Pro Met Ile
485 490 495
Ile Thr Lys Gly Ser Asp Gln Val Ala Ile Ser Ser Arg Phe Glu Ile
500 505 510
Arg Asn Asp Pro Ser Ile Val Arg Asn Tyr Gly Ser Met Leu Val Glu
515 520 525
Phe Ala Lys Ile Thr Pro Asp Gly Met Val Val Phe Phe Pro Ser Tyr
530 535 540
Leu Tyr Met Glu Ser Ile Val Ser Met Trp Gln Thr Met Gly Ile Leu
545 550 555 560
Asp Glu Val Trp Lys His Lys Leu Ile Leu Val Glu Thr Pro Asp Ala
565 570 575
Gln Glu Thr Ser Leu Ala Leu Glu Thr Tyr Arg Lys Ala Cys Ser Asn
580 585 590
Gly Arg Gly Ala Ile Leu Leu Ser Val Ala Arg Gly Lys Val Ser Glu
595 600 605
Gly Ile Asp Phe Asp His Gln Tyr Gly Arg Thr Val Leu Met Ile Gly
610 615 620

Ile Pro Phe Gln Tyr Thr Glu Ser Arg Ile Leu Lys Ala Arg Leu Glu
625 630 635 640
Phe Met Arg Glu Asn Tyr Arg Ile Arg Glu Asn Asp Phe Leu Ser Phe
645 650 655
Asp Ala Met Arg His Ala Ala Gln Cys Leu Gly Arg Val Leu Arg Gly
660 665 670
Lys Asp Asp Tyr Gly Val Met Val Leu Ala Asp Arg Arg Phe Ser Arg
675 680 685
Lys Arg Ser Gln Leu Pro Lys Trp Ile Ala Gln Gly Leu Ser Asp Ala
690 695 700
Asp Leu Asn Leu Ser Thr Asp Met Ala Ile Ser Asn Thr Lys Gln Phe
705 710 715 720
Leu Arg Thr Met Ala Gln Pro Thr Asp Pro Lys Asp Gln Glu Gly Val
725 730 735
Ser Val Trp Ser Tyr Glu Asp Leu Ile Lys His Gln Asn Ser Arg Lys
740 745 750
Asp Gln Gly Gly Phe Ile Glu Asn Glu Asn Lys Glu Gly Glu Gln Asp
755 760 765
Glu Asp Glu Asp Glu Asp Ile Glu Met Gln
770 775

<210> SEQ ID NO:37
<211> LENGTH: 772
<212> TYPE: PRT
<213> ORGANISM:S. pombe

<400> SEQ ID NO:37
Met Lys Phe Tyr Ile Asp Asp Leu Pro Ile Leu Phe Pro Tyr Pro Arg
1 5 10 15
Ile Tyr Pro Glu Gln Tyr Gln Tyr Met Cys Asp Leu Lys His Ser Leu
20 25 30
Asp Ala Gly Gly Ile Ala Leu Leu Glu Met Pro Ser Gly Thr Gly Lys
35 40 45
Thr Ile Ser Leu Leu Ser Leu Ile Val Ser Tyr Gln Gln His Tyr Pro
50 55 60
Glu His Arg Lys Leu Ile Tyr Cys Ser Arg Thr Met Ser Glu Ile Asp
65 70 75 80
Lys Ala Leu Ala Glu Leu Lys Arg Leu Met Ala Tyr Arg Thr Ser Gln
85 90 95
Leu Gly Tyr Glu Glu Pro Phe Leu Gly Leu Gly Leu Thr Ser Arg Lys
100 105 110
Asn Leu Cys Leu His Pro Ser Val Arg Arg Glu Lys Asn Gly Asn Val
115 120 125
Val Asp Ala Arg Cys Arg Ser Leu Thr Ala Gly Phe Val Arg Glu Gln
130 135 140
Arg Leu Ala Gly Met Asp Val Pro Thr Cys Glu Phe His Asp Asn Leu
145 150 155 160
Glu Asp Leu Glu Pro His Ser Leu Ile Ser Asn Gly Val Trp Thr Leu
165 170 175
Asp Asp Ile Thr Glu Tyr Gly Glu Lys Thr Thr Arg Cys Pro Tyr Phe
180 185 190
Thr Val Arg Arg Met Leu Pro Phe Cys Asn Val Ile Ile Tyr Ser Tyr
195 200 205
His Tyr Leu Leu Asp Pro Lys Ile Ala Glu Arg Val Ser Arg Glu Leu
210 215 220
Ser Lys Asp Cys Ile Val Val Phe Asp Glu Ala His Asn Ile Asp Asn
225 230 235 240
Val Cys Ile Glu Ser Leu Ser Ile Asp Leu Thr Glu Ser Ser Leu Arg
245 250 255

Lys Ala Ser Lys Ser Ile Leu Ser Leu Glu Gln Lys Val Asn Glu Val
260 265 270

Lys Gln Ser Asp Ser Lys Lys Leu Gln Asp Glu Tyr Gln Lys Leu Val
275 280 285

Arg Gly Leu Gln Asp Ala Asn Ala Ala Asn Asp Glu Asp Gln Phe Met
290 295 300

Ala Asn Pro Val Leu Pro Glu Asp Val Leu Lys Glu Ala Val Pro Gly
305 310 315 320

Asn Ile Arg Arg Ala Glu His Phe Ile Ala Phe Leu Lys Arg Phe Val
325 330 335

Glu Tyr Leu Lys Thr Arg Met Lys Val Leu His Val Ile Ala Glu Thr
340 345 350

Pro Thr Ser Phe Leu Gln His Val Lys Asp Ile Thr Phe Ile Asp Lys
355 360 365

Lys Pro Leu Arg Phe Cys Ala Glu Arg Leu Thr Ser Leu Val Arg Ala
370 375 380

Leu Gln Ile Ser Leu Val Glu Asp Phe His Ser Leu Gln Gln Val Val
385 390 395 400

Ala Phe Ala Thr Leu Val Ala Thr Tyr Glu Arg Gly Phe Ile Leu Ile
405 410 415

Leu Glu Pro Phe Glu Thr Glu Asn Ala Thr Val Pro Asn Pro Ile Leu
420 425 430

Arg Phe Ser Cys Leu Asp Ala Ser Ile Ala Ile Lys Pro Val Phe Glu
435 440 445

Arg Phe Arg Ser Val Ile Ile Thr Ser Gly Thr Leu Ser Pro Leu Asp
450 455 460

Met Tyr Pro Lys Met Leu Gln Phe Asn Thr Val Met Gln Glu Ser Tyr
465 470 475 480

Gly Met Ser Leu Ala Arg Asn Cys Phe Leu Pro Met Val Val Thr Arg
485 490 495

Gly Ser Asp Gln Val Ala Ile Ser Ser Lys Phe Glu Ala Arg Asn Asp
500 505 510

Pro Ser Val Val Arg Asn Tyr Gly Asn Ile Leu Val Glu Phe Ser Lys
515 520 525

Ile Thr Pro Asp Gly Leu Val Ala Phe Phe Pro Ser Tyr Leu Tyr Leu
530 535 540

Glu Ser Ile Val Ser Ser Trp Gln Ser Met Gly Ile Leu Asp Glu Val
545 550 555 560

Trp Lys Tyr Lys Leu Ile Leu Val Glu Thr Pro Asp Pro His Glu Thr
565 570 575

Thr Leu Ala Leu Glu Thr Tyr Arg Ala Ala Cys Ser Asn Gly Arg Gly
580 585 590

Ala Val Leu Leu Ser Val Ala Arg Gly Lys Val Ser Glu Gly Val Asp
595 600 605

Phe Asp His His Tyr Gly Arg Ala Val Ile Met Phe Gly Ile Pro Tyr
610 615 620

Gln Tyr Thr Glu Ser Arg Val Leu Lys Ala Arg Leu Glu Phe Leu Arg
625 630 635 640

Asp Thr Tyr Gln Ile Arg Glu Ala Asp Phe Leu Thr Phe Asp Ala Met
645 650 655

Arg His Ala Ala Gln Cys Leu Gly Arg Val Leu Arg Gly Lys Asp Asp
660 665 670

His Gly Ile Met Val Leu Ala Asp Lys Arg Tyr Gly Arg Ser Asp Lys
675 680 685

Arg Thr Lys Leu Pro Lys Trp Ile Gln Gln Tyr Ile Thr Glu Gly Ala
690 695 700

Thr Asn Leu Ser Thr Asp Met Ser Leu Ala Leu Ala Lys Lys Phe Leu
705 710 715 720

Arg Thr Met Ala Gln Pro Phe Thr Ala Ser Asp Gln Glu Gly Ile Ser
725 730 735

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Trp Trp Ser Leu Asp Asp Leu Leu Ile His Gln Lys Lys Ala Leu Lys
            740                 745                 750
Ser Ala Ala Ile Glu Gln Ser Lys His Glu Asp Glu Met Asp Ile Asp
        755                 760                 765
Val Val Glu Thr
    770

<210> SEQ ID NO:38
<211> LENGTH: 760
<212> TYPE: PRT
<213> ORGANISM:Homo sapien

<400> SEQ ID NO:38
Met Lys Leu Asn Val Asp Gly Leu Leu Val Tyr Phe Pro Tyr Asp Tyr
 1               5                  10                  15
Ile Tyr Pro Glu Gln Phe Ser Tyr Met Arg Glu Leu Lys Arg Thr Leu
            20                  25                  30
Asp Ala Lys Gly His Gly Val Leu Glu Met Pro Ser Gly Thr Gly Lys
        35                  40                  45
Thr Val Ser Leu Leu Ala Leu Ile Met Ala Tyr Gln Arg Ala Tyr Pro
    50                  55                  60
Leu Glu Val Thr Lys Leu Ile Tyr Cys Ser Arg Thr Val Pro Glu Ile
65                  70                  75                  80
Glu Lys Val Ile Glu Glu Leu Arg Lys Leu Leu Asn Phe Tyr Glu Lys
                85                  90                  95
Gln Glu Gly Glu Lys Leu Pro Phe Leu Gly Leu Ala Leu Ser Ser Arg
            100                 105                 110
Lys Asn Leu Cys Ile His Pro Glu Val Thr Pro Leu Arg Phe Gly Lys
        115                 120                 125
Asp Val Asp Gly Lys Cys His Ser Leu Thr Ala Ser Tyr Val Arg Ala
    130                 135                 140
Gln Tyr Gln His Asp Thr Ser Leu Pro His Cys Arg Phe Tyr Glu Glu
145                 150                 155                 160
Phe Asp Ala His Gly Arg Glu Val Pro Leu Pro Ala Gly Ile Tyr Asn
                165                 170                 175
Leu Asp Asp Leu Lys Ala Leu Gly Arg Arg Gln Gly Trp Cys Pro Tyr
            180                 185                 190
Phe Leu Ala Arg Tyr Ser Ile Leu His Ala Asn Val Val Val Tyr Ser
        195                 200                 205
Tyr His Tyr Leu Leu Asp Pro Lys Ile Ala Asp Leu Val Ser Lys Glu
    210                 215                 220
Leu Ala Arg Lys Ala Val Val Val Phe Asp Glu Ala His Asn Ile Asp
225                 230                 235                 240
Asn Val Cys Ile Asp Ser Met Ser Val Asn Leu Thr Arg Arg Thr Leu
                245                 250                 255
Asp Arg Cys Gln Gly Asn Leu Glu Thr Leu Gln Lys Thr Val Leu Arg
            260                 265                 270
Ile Lys Glu Thr Asp Glu Gln Arg Leu Arg Asp Glu Tyr Arg Arg Leu
        275                 280                 285
Val Glu Gly Leu Arg Glu Ala Ser Ala Ala Arg Glu Thr Asp Ala His
    290                 295                 300
Leu Ala Asn Pro Val Leu Pro Asp Glu Val Leu Gln Glu Ala Val Pro
305                 310                 315                 320
Gly Ser Ile Arg Thr Ala Glu His Phe Leu Gly Phe Leu Arg Arg Leu
                325                 330                 335
Leu Glu Tyr Val Lys Trp Arg Leu Arg Val Gln His Val Val Gln Glu
            340                 345                 350
Ser Pro Pro Ala Phe Leu Ser Gly Leu Ala Gln Arg Val Cys Ile Gln
        355                 360                 365
Arg Lys Pro Leu Arg Phe Cys Ala Glu Arg Leu Arg Ser Leu Leu His
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        370                 375                 380

Thr Leu Glu Ile Thr Asp Leu Ala Asp Phe Ser Pro Leu Thr Leu Leu
385                 390                 395                 400
Ala Asn Phe Ala Thr Leu Val Ser Thr Tyr Ala Lys Gly Phe Thr Ile
                405                 410                 415
Ile Ile Glu Pro Phe Asp Asp Arg Thr Pro Thr Ile Ala Asn Pro Ile
            420                 425                 430
Leu His Phe Ser Cys Met Asp Ala Ser Leu Ala Ile Lys Pro Val Phe
        435                 440                 445
Glu Arg Phe Gln Ser Val Ile Ile Thr Ser Gly Thr Leu Ser Pro Leu
    450                 455                 460
Asp Ile Tyr Pro Lys Ile Leu Asp Phe His Pro Val Thr Met Ala Thr
465                 470                 475                 480
Phe Thr Met Thr Leu Ala Arg Val Cys Leu Cys Pro Met Ile Ile Gly
                485                 490                 495
Arg Gly Asn Asp Gln Val Ala Ile Ser Ser Lys Phe Glu Thr Arg Glu
            500                 505                 510
Asp Ile Ala Val Ile Arg Asn Tyr Gly Asn Leu Leu Leu Glu Met Ser
        515                 520                 525
Ala Val Val Pro Asp Gly Ile Val Ala Phe Phe Thr Ser Tyr Gln Tyr
    530                 535                 540
Met Glu Ser Thr Val Ala Ser Trp Tyr Glu Gln Gly Ile Leu Glu Asn
545                 550                 555                 560
Ile Gln Arg Asn Lys Leu Leu Phe Ile Glu Thr Gln Asp Gly Ala Glu
                565                 570                 575
Thr Ser Val Ala Leu Glu Lys Tyr Gln Glu Ala Cys Glu Asn Gly Arg
            580                 585                 590
Gly Ala Ile Leu Leu Ser Val Ala Arg Gly Lys Val Ser Glu Gly Ile
        595                 600                 605
Asp Phe Val His His Tyr Gly Arg Ala Val Ile Met Phe Gly Val Pro
    610                 615                 620
Tyr Val Tyr Thr Gln Ser Arg Ile Leu Lys Ala Arg Leu Glu Tyr Leu
625                 630                 635                 640
Arg Asp Gln Phe Gln Ile Arg Glu Asn Asp Phe Leu Thr Phe Asp Ala
                645                 650                 655
Met Arg His Ala Ala Gln Cys Val Gly Arg Ala Ile Arg Gly Lys Thr
            660                 665                 670
Asp Tyr Gly Leu Met Val Phe Ala Asp Lys Arg Phe Ala Arg Gly Asp
        675                 680                 685
Lys Arg Gly Lys Leu Pro Arg Trp Ile Gln Glu His Leu Thr Asp Ala
    690                 695                 700
Asn Leu Asn Leu Thr Val Asp Glu Gly Val Gln Val Ala Lys Tyr Phe
705                 710                 715                 720
Leu Arg Gln Met Ala Gln Pro Phe His Arg Glu Asp Gln Leu Gly Leu
                725                 730                 735
Ser Leu Leu Ser Leu Glu Gln Leu Glu Ser Glu Glu Thr Leu Lys Arg
            740                 745                 750
Ile Glu Gln Ile Ala Gln Gln Leu
        755                 760
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/33065

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12N 9/00, 9/10, 1/20; C12N 15/00; C07H 21/02, 21/04
US CL :435/183, 193, 252.3, 320.1, 6; 536/23.1, 23.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/183, 193, 252.3, 320.1, 6; 536/23.1, 23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST, STN, Medline, CAPLUS, BIOSIS, JAPIO, PATOSWO, PATOSEP, SCISEARCH, EMBASE, search terms, helicase, NHL protein, mammalian, human, RAD3/ERCC2 gene family, SEQ ID NOs : 1 and 2

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,843,737 A (CHEN et al) 01 December 1998, see entire document.	1
X, P	BAI et al, Overexpression of M68/DcR3 in human gastrointestinal tract tumors independent of gene amplification and its location in four-gene cluster. Proc. Natl. Acad. Sci. USA. 01 Febuary 2000. Vol 97. No. 3, pages 1230-1235.	1-26
X	US 5,888,792 A (BANDMAN et al) 30 March 1999, see entire document.	1
Y, P	ZHOU et al. Pif1p Helicase, a Catalytic Inhibitor of Telomerase in Yeast. Science. 04 August 2000. Vol.289. pages 771-774.	1

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 MARCH 2001	19 APR 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer PONNATHAPURA ACHUTAMURTHY
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/33065

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,466,576 A (SCHULZ et al) 14 November 1995, see entire document.	1-26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/33065

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-22 and 26, drawn to a purified DNA molecule encoding a mammalian NHL protein, vectors and host cells comprising said DNA, methods of expressing said DNA and the NHL protein.
Group II, claim(s) 23-25, drawn to an isolated molecule which comprises the nucleotide sequence as set forth in SEQ ID NO: 3.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The technical relationship shared between the claims of groups I and II corresponds to a DNA molecule encoding a mammalian NHL (novel helicase-like) protein. Chen et al. (US Patent No: 5,843,737) teach a gene that encodes a multifunctional protein having helicase activity and hence the inventions do not share a special technical feature.